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A LIMNOLOGICAL STUDY OF THE PROFUNDAL BOTTOM FAUNA OF CERTAIN FRESH-WATER LAKES

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CONTENTS

A LIMNOLOGICAL STUDY OF THE PROFUNDAL BOTTOM FAUNA OF CERTAIN FRESH-WATER LAKES*

INTRODUCTION

In fresh-water lakes few animals have proved themselves capable of living in that unusual habitat, the anaerobic profundal zone of the lake bottom. Although this qualitatively limited fauna is ordinarily exposed to continuously low temperatures, little or no light, a pH often falling below neutrality, relatively large amounts of free carbon dioxide, a total lack of dissolved oxygen for months at a time and in some instances the accumulation of certain decomposition gases, it nevertheless comprises a population of bottom-dwelling animals frequently amounting to many thousand individuals per square meter.

It is the purpose of this paper to present the results from a study of the macroscopic profundal bottom fauna in which the principal emphasis has been placed on the ecological relations of the animals involved rather than on the qualitative and quantitative aspects alone, as has been so frequently done in the past. Aquatic productivity is certainly to be measured in terms of quality and quantity. However, to stop there is to leave a large and fundamentally important part in the complete problem of biological productivity wholly untouched. The aim throughout this investigation has been to discover not only the nature, quantity and distribution of the bottom fauna beneath the deeper waters, but having done that, to study and measure the environmental influences in an attempt to evaluate their limnological importance. During the work, the writer has become convinced of the need for great caution in pointing out any single factor as the sole determining influence in the abundance and distribution of bottom animals.

Field work was begun in February, 1923, and discontinued in the third week of August for that year. During the summer of 1925, field studies occupied about three weeks, while in June, 1926, the work was again taken up and has been in continuous progress, with only minor interruptions, since that time. The last field records included are for February 9, 1929.

The major portion of this investigation was conducted on two Michigan lakes, Douglas and Third Sister. Several other Michigan and New York lakes were studied and data from one of these (Kirkville Green Lake in New York) are included in this paper.

The total number of bottom samples, 1879, were obtained as follows: 1331 from Douglas Lake, of which 1007 came from the profundal zone

* Contribution from the University of Michigan Biological Station and from the Zoölogical Laboratory, University of Michigan.

within the six major depressions and the remaining 324 from numerous sampling series extending from the deepest water as far up the slope as any significant numbers of profundal animals were found; 403 from Third Sister, 163 of these being from below the 18 m. contour; and 145 from the deeper water of Kirkville Green Lake.

In addition to these qualitative and quantitative bottom samples, more than 180 vertical series of physical-chemical determinations have been made; a considerable amount of experimental work conducted in the lakes studied and in the laboratory; some further physical analyses made; certain original hydrographic and morphometric work done and a large amount of general ecological data relating to the problem collected. Obviously, not all this mass of data in its original detail can be included here, and, in fact, that which is presented is very largely in summarized form. Details are given only where necessary for clarity.

METHODS AND EQUIPMENT

Physical

Morphometric data have been derived from various sources. Some were from original work and, in such instances, surveying compasses, surveyor's transits, and steel tapes constituted the equipment. In studying the maps, areas were determined by a polar planimeter; shore and contour lengths by a standard map measurer.

Certain determinations of relative transparency were made, using a Secchi's disc under standard conditions. Temperatures were determined with Negretti-Zambra reversing thermometers.

For physical examination and analysis of bottom deposits, samples were collected with an Ekman-Birge dredge (Ekman, 1911; Birge, 1922). After collection, the samples were put through a set of 6 Tyler Standard Scale Screens with mesh openings of 4.7, 2.4, 1.2, 0.4, 0.2, and 0.15 mm., respectively, to determine the composition and fineness of the mud. For determining amounts of water and of volatile and non-volatile solids present in bottom mud, use was made of heavy analytical balances for weighing the wet mud; electric oven for drying; fused silica crucibles and gas blast lamp for ignition; and, finally, Chainomatic analytical balances for determining loss on ignition.

Chemical

Hydrogen ion concentration was determined colorimetrically in the field at the time of sampling. Sets of color standard tubes prepared by the manufacturer and standard indicators were purchased from La Motte Chemical Products Co. A new series was secured annually. Regular and frequent checking of such sets both electrometrically and against duplicate tubes was a regular procedure.

Dissolved oxygen was determined by the Rideal-Stewart modification of the Winkler method as given by Standard Methods of Water Analysis. Samples collected in the field were at once treated with the reagents up to the final titration point and stored in covered cases until the laboratory had been reached. Final titrations were always made within 6 hours, usually much less, from the time of collection. Frequent standardization of reagents was routine practice.

For free carbon dioxide determinations, N/44 KOH and phenolphthalein were employed. These analyses were always made as quickly as possible after the sample had been brought into the boat. Phenolphthalein alkalinity and methyl orange alkalinity titrations have also been part of the regular work. In each of these determinations the procedure described in the previously mentioned Standard Methods was followed.

Water for all chemical analyses was collected with the modified Kemmerer Water Bottle (Birge, 1922, pp. 547-549, Pl. XL, Figs. 6-7). Portable chemical, instrument and sample bottle kits, designed by Professor Paul S. Welch have made possible accurate physical-chemical determinations in the field, with the exception of final titrations as noted.

Biological

All bottom samples were taken with a small Ekman-Birge dredge which collected a sample of 225 sq. cm. surface area. In the soft mud bottom of most lakes studied, the dredge always came up filled to the top. Usually, however, the upper layer was composed of "dust-fine detritus" in suspension and the lower part of more solid mud. On firmer but still definitely muddy bottoms, the dredge penetrated to a depth of 5-10 cm. With the increase of sand or fine gravel in the mud the dredge worked less and less satisfactorily, until it failed in about 75 per cent of the trials on sandy bottoms.

Usually at least 5 hauls were made with the dredge and placed together as a sample from a particular depth or station. Frequently, 10 or 15 and sometimes as many as 50 hauls were thus combined into one sample. Care was exercised, however, to see that they were truly random samples within the area studied.

The mud was emptied from the dredge into a pail, stirred with the hand to reduce it to a uniform consistency, and then transferred to a deep pan, 50 cm. in diameter, which had a bottom composed of brass screening with a mesh fine enough to retain the smallest macroscopic organisms.

The captured animals, together with the coarse debris, left in the screen after the mud had been removed by a thorough washing in the lake, were at once transferred to wide-mouthed bottles and enough water added to keep them alive until the laboratory had been reached. There they were sorted and counted. Much of the material was either preserved in vials or used for experiments. Some insect larvae and pupae were isolated and reared. Many

were placed in rearing cages, each of which usually contained representatives of but one genus. Such cages were constructed of two parts, the lower halves being glass-sided, slate-bottomed, rectangular aquaria provided with a layer of mud and a supply of running lake water while the upper halves were constructed of a wire framework shaped like the aquaria and inverted over them but covered with fine mesh cotton screening.

For determining the number of Corethra larvae at different levels in the lake, a closing plankton net (Juday, 1916, pp. 573-575, Pl. XXXIV, Figs. 1-3) equipped with No. 6, standard quality bolting cloth, was used. This size of mesh (28 per cm.) caught even the youngest larvae, when present, and yet by reason of its lower resistance to the water it increased the efficiency considerably over a net with No. 20 bolting cloth.

Regular procedure when making physical-chemical determinations and qualitative-quantitative bottom fauna collections was as follows:

1. A complete vertical series of temperature readings.
2. Samples for dissolved oxygen at intervals selected according to conditions of temperature distribution.
3. Determinations of pH and free carbon dioxide with freshly collected samples during the digestion period required in the oxygen analyses.
4. Collection of another set of samples for determining phenolphthalein and methyl orange alkalinity.
5. If on the program for the day, vertical catches with a closing plankton net to determine the presence or absence of Corethra larvae in the water.
6. Bottom sampling.

Experimental

Considerable experimental work was carried on in the lakes themselves and for this purpose it became necessary to sink certain apparatus to the lake bottom. A plan for supporting experimental boxes on the lake floor, or at any desired level in the lake, was devised in the spring of 1927 (Fig. 62). Perhaps the most important advantage of this arrangement is the possibility of raising and examining any one experimental box without disturbing the others or the anchors. In these experiments, use has been made of galvanized wire baskets as receptacles for holding the various glass containers in which experimental animals were placed. Further details of the experiments and their conduct will be found in the section where the results are discussed.

Besides the experiments on the lake floor, a certain amount of such work has been done in the laboratory, some of which involved the use of various constant temperature devices. Low temperatures were secured by using a circulating brine system and high temperatures by employing a DeKotinsky

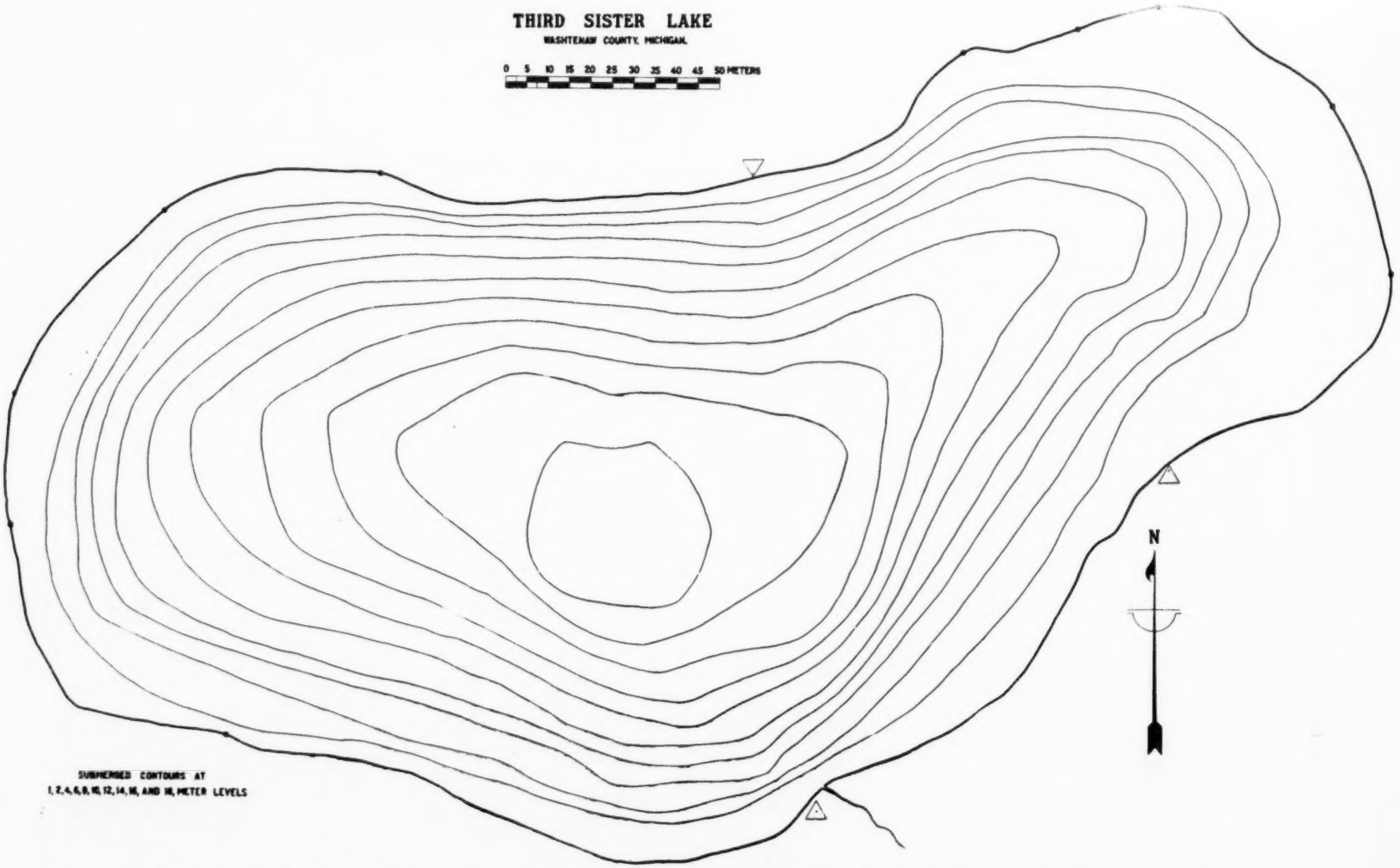


FIG. 1. Map of Third Sister Lake. The small triangles on shore indicate position of permanent monuments over which the transits were located. The dot within the triangle indicates the exact location of the monument.

Constant Temperature Apparatus. During the toleration experiments (Fig. 63) tanks of compressed oxygen, hydrogen and carbon dioxide were used.

PHYSIOGRAPHY AND MORPHOMETRY

Third Sister Lake

West of Ann Arbor, Washtenaw County, Michigan, are three small bodies of water known as the Three Sister Lakes. The largest of the group, Third Sister, is the only one with which this report is concerned. It is deep for its size, stratifies thermally in summer, and drains into the Great Lakes-St. Lawrence system through the Huron River. The latter stream enters Lake Erie near its northwestern corner.

Third Sister Lake has two possible sources of inflowing water and one certain, though intermittent, one. There is the possibility that part of the water coming into the lake reaches it through springs on its floor and the apparently stronger possibility that seepage from bordering boggy areas and surrounding higher ground adds more water, while every heavy rain transforms the, at other times dry, creek entering on the south into a raging little torrent. The heavy loads of suspended substances which this small stream brings down from the cultivated fields on the adjacent kames play an important part in the character and the rate and mode of deposition on the lake bottom.

There is a single outlet flowing through a small marsh southwest of the lake. About one-fourth of the shore is firm and composed of sandy gravel; the remainder is very soft and muddy with a semi-floating mat of sphagnum bordering the water line along part of the western end. Mixed with the sphagnum are many other plants typical of such situations. The submerged and emergent vegetation zone extends approximately to the 3 m. depth.

When work was begun, only an outline map of Third Sister Lake was available. To complete the study and to form the basis for computations, a detailed and accurate hydrographic map became a necessity. The instruments used were two transits and one surveying compass. In mapping the shore line, 60 positions were chosen; bearings taken on them by each of the three instruments; and the angles later protracted. To check on the outline thus secured, 10 points, marked by small solid circles on the shore line of the map (Fig. 1), were determined by actual measurement with steel tape over the ice in mid-winter. Computations based on a large map, scale of 1:250, showed that differences between results secured by the two methods in no case exceeded 0.15 m. For present purposes this is sufficiently accurate since fluctuations of water level exceed 0.25 m. Computed and measured lengths of the base lines were also checked in the same manner and found to agree more closely, the greatest discrepancy between results secured by the two methods being about 0.1 m. Submerged contours are based on approximately 200 soundings.

The only morphometric data on Third Sister Lake which the writer has seen in print are contained in a paper by Weld (1904, p. 38). This author gives the elevation of the three lakes in the group as "approximately the same, *i.e.*, 914 ft. (278 m.) above sea level" and states on the same page, that, "The depth of the lakes varies with the size, the first being 18 ft. (5.5 m.) deep . . . and the third 55 ft. (16.75 m.)." Evidently the deepest part of the lake had not been found at that time, since a considerable area is 18 m. or more in depth.

Maximum length	310 m.
Maximum breadth	150 m.
Maximum depth	18.5 m.
Mean depth[1]	7.35 m.
Direction of main axis	northeast and southwest
Area	38,500 sq.m.
Elevation	278 m.
Length of shore line	863 m.
Shore development	1.24
Volume	283,153 cu. m.
Volume development	1.22
Mean slope of bottom[2]	1.3%

There is but one depression, nearly centrally located, in this lake. The general shape of the basin is that of a scoop, being shallow in the eastern portion, quite generally deeper in the western end and deepest somewhat west of the center. The slope of the bottom is gentle in the eastern part and moderately steep along the southern and western sides.

TABLE I. *Areas and volumes of the water at various levels Third Sister Lake.*

Depth meters	Areas		Stratum meters	Vol. each stratum cu. m.	% of Total vol.	Underlying vol. in % of Total
	Sq. meters	% of surface				
0.....	38,500	100	0–1	34,612	12.22	87.78
1.....	30,866	80.2	1–2	28,996	10.24	77.54
2.....	27,166	70.6	2–4	50,705	17.90	59.64
4.....	23,582	61.3	4–6	44,250	15.63	44.01
6.....	20,700	53.8	6–8	37,759	13.34	30.67
8.....	17,116	44.4	8–10	30,456	10.76	19.91
10.....	13,416	34.8	10–12	23,296	8.23	11.68
12.....	9,966	25.9	12–14	16,879	5.96	5.72
14.....	7,000	18.2	14–16	11,045	3.90	1.82
16.....	4,167	10.8	16–18	5,155	1.82	0.00
18.....	1,267	3.3				

[1] Also called "reduced thickness."
[2] The figures indicated by footnote references 1 and 2 were computed with formulae given by Juday (1914; p. 122).

TABLE II. *Areas of the bottom at various levels Third Sister Lake.*

Depth meters	Length of contour meters	Stratum meters	Area between contours sq. meters	% of total bottom area
0	863	0–1	7,634	19.8
1	750	1–2	3,700	9.6
2	713	2–4	3,584	9.3
4	680	4–6	2,882	7.5
6	643	6–8	3,584	9.3
8	595	8–10	3,700	9.6
10	518	10–12	3,450	9.0
12	425	12–14	2,966	7.7
14	345	14–16	2,833	7.4
16	263	16–18	2,900	7.5
18	135	18–18.5	1,267	3.3
Totals	—	———	38,500	100.0

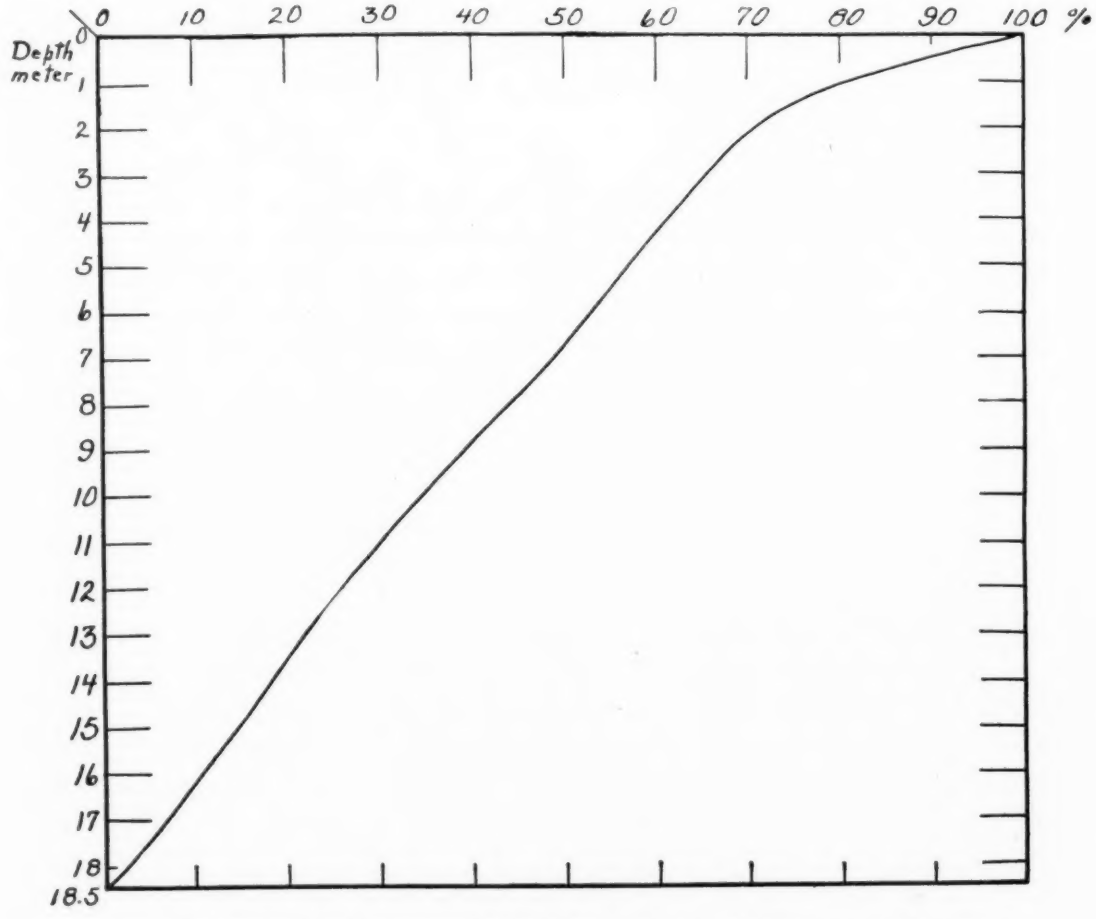


FIG. 2. Curve showing area of lake basin at any depth as a per cent of area of surface.

FIG. 3. Map of Douglas Lake. Position of the seven depressions, maximum depth of each in meters, station number of each depression, and extent of the profundal (15) and the sublittoral (9) zones are indicated as follows: F. I., 28 m., S30, Fairy Island depression, maximum depth 28 m., Station 30; R. P., 22 m., S50, Roberts Point depression, maximum depth 22 m., Station 50; G.V.P., 25 m., S 10, Grapevine Point depression, maximum depth 25 m., Station 10; ST.P., 19 m., S40, Stony Point depression, maximum depth 19 m., Station 40; SE.P., 24 m., S20, Sedge Point depression, maximum depth 24 m., Station 20; S.F-T., 24 m., S1, South Fish-Tail depression, maximum depth 24 m., Station 1; M.P., Maple Point depression. The area included within the 15 meter contour indicates profundal zone, that between 9 and 15 meters the sublittoral, and the region between the shore line and the 9 meter contour represents the extent of the littoral zone.

Douglas Lake

This body of water is located near the northern tip of the southern peninsula, Michigan, approximately 15 miles south of the Straits of Mackinac and about midway between Cheboygan on Lake Huron and Petosky on Lake Michigan. It is a moderately deep lake of glacial origin, showing typical thermal stratification, with certain exceptions discussed by Welch (1927). It empties into Lake Huron through the Cheboygan River drainage. Scott (1921, pp. 106-117) discussed the geological history and physiography of Douglas Lake and Welch (1927) has given in summarized form a large amount of morphometric data. In the present paper, areas of certain subdivisions of the lake bottom are given in greater detail. The hydrographic map from which this information was derived and from which data have been taken for constructing the outline map of the lake included herein, was made by the Department of Geodesy and Surveying, University of Michigan.

None of the depths given in this paper are referred to standard low water datum but all are given as average depths recorded in various depressions and stations at the time of sampling. Thus the maximum depth of Douglas Lake, standard low water datum, is 89 ft. (27.1 m.), but throughout this paper the greater depth of 28 m. is used. This procedure seems preferable

TABLE III. *Area of bottom at various levels in the six major depressions and in the inter-depression regions of Douglas Lake.*

Depression	Depth meters	Length of contour meters	Strata meters	Area between contours square meters	% total bottom area
South Fish-Tail*.....	9.14	2,784	9–15	128,636	0.85
	15.24	2,304	15–24	246,515	1.63
Grapevine Point.....	9.14	2,832	9–15	232,121	1.53
	15.24	2,448	15–25	181,818	1.20
Sedge Point.....	9.14	6,480	9–15	1,187,878	7.87
	15.24	1,392	15–24	124,091	0.82
Fairy Island.....	9.14	4,992	9–15	290,909	1.93
	15.24	3,648	15–28	562,424	3.72
Stony Point.....	9.14	1,920	9–15	116,818	0.77
	15.24	960	15–19	35,000	0.23
Roberts Point.....	9.14	2,256	9–15	93,939	0.62
	15.24	1,776	15–22	214,545	1.42
Whole Lake.....	0.0	25,123	0–9	11,517,733	76.27
	9.14	23,808	9–15	2,206,210	14.62
	15.24	13,056	15–28	1,375,757	9.11

9.14 meters (30 feet)
15.24 meters (50 feet)

*In earlier work on Douglas Lake, e.g. (Welch, 1927) this region of the lake has been called "Camp Davis depression." Since that time the name has been officially changed to "South Fish-Tail depression."

for the purposes of this particular study since it is depth of water at a given time and not the lowest level recorded which is of importance to the bottom animals then being studied.

One very distinctive feature of Douglas Lake is the peculiarity of its basin. Scott *(loc. cit.)* calls attention to the apparent division of the lake into three basins and Welch (1927) further discusses the lake floor and points out that actually there are seven distinct basins or depressions in the lake aside from the deeper water of North Fish-Tail Bay. It is further pointed out by the latter author that these depressions are isolated from each

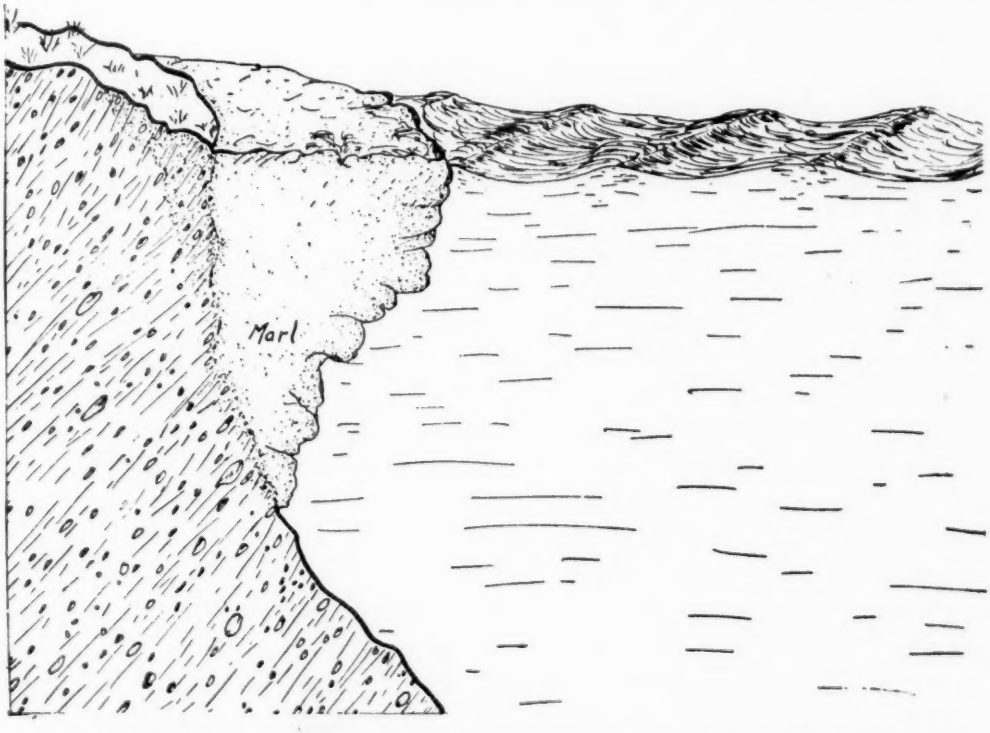


FIG. 4. Diagrammatic sketch showing marl shelf on shore of Kirkville Green Lake.

other by depth contours of 40 ft. (12.19 m.) or less and that they behave, in a physical-chemical sense, as separate lakes. The biological importance of this phenomenon will receive some discussion in this paper.

Kirkville Green Lake

Kirkville Green Lake is located in Onondaga County, New York, approximately 1.5 miles southwest of Kirkville, 10 miles east of Syracuse and about 9 miles south of Oneida Lake, into which it empties through a tributary of Chittenango Creek. It is thus within the Great Lakes water-shed. The lake is scarcely more than 0.5 mile long but attains the surprising depth of 61 m. Due to this, and to the fact that it is unusually well protected from wind in all but one direction, it exhibits certain physical, chemical and biological features which make it very interesting.

Hardly less remarkable than the intense bottle-green color of the water over the depths is the size of the marl deposits along the banks and on the lake floor. The writer has taken marl incrustations 10 cm. thick from a depth of 2-3 m. Frequently, cylindrical incrustations, 15-16 cm. in diameter, were brought up which, when broken open, were found to be deposited upon a twig perhaps 5 mm. in diameter. Solid banks of marl occur along shore and in some places these appear to be several meters thick. In at least one place an overhanging shelf of creamy-white marl has been built out from shore with its upper surface just beneath the water level and its edge more than 2.5 m. from the bank. This shelf tapers back at its base and thus has the form shown in diagrammatical cross-section in Fig. 4.

Except for a narrow beach extending across the northern end of the lake, there is no submerged shelf and the bottom drops precipitously into deep water from the shore line. It is possible to stand on the marl deposit extending out from the bank at one point on shore and drop a sounding lead over the edge to a depth of 18 m.

It should be pointed out that none of the lakes studied are in any sense contaminated or polluted bodies of water. At least one has been considered as a source of water supply for a neighboring city. Each is as nearly unmodified as a lake is apt to be in settled regions. There are no industrial wastes emptied within the area drained by any of them and in the case of one there are no dwellings within 0.5 mile of its shores. Douglas Lake has a few summer colonies and the University of Michigan Biological Station on its shores, and the others are bordered by farm lands.

ACKNOWLEDGMENTS

Many people have assisted the writer in various ways during this investigation and such service is here gratefully acknowledged. The work has been done under the general direction of Professor Paul S. Welch. To Director George R. LaRue, the writer is indebted for the use of facilities at the University of Michigan Biological Station. Thanks are also due to Dean Samuel T. Dana and other staff members in the University of Michigan School of Forestry and Conservation for many courtesies extended to the author in connection with the study of Third Sister Lake; to Professor William M. Smallwood and the Department of Zoölogy, Syracuse University, for the use of facilities while work was in progress on Kirkville Green Lake, and to the following authorities for identification of specimens: Professor O. A. Johannsen, *Chironomidae* and *Culicidae*, both immature and adults; Professor Frank Smith, *Oligochaeta;* Dr. V. Sterki, *Sphaeriidae;* Dr. Bryant Walker, *Gastropoda;* Dr. Ruth Marshall, *Hydracarina;* Dr. J. Percy Moore, *Hirudinea;* Dr. L. J. Thomas; *Mermithidae;* and Dr. N. A. Cobb, free-living *Nematoda.*

PROFUNDAL BENTHIC HABITAT

General Statement

Ecologically, a lake may be divided into two regions, the limnetic and the benthic. Each has its own set of peculiarly characteristic phenomena. The physical, chemical, and biological factors of the lake bottom are, moreover, far from uniform from shore to lake center and this lack of uniformity results both in the need for and the means of further subdivision and a classification of benthic habitats.

Classification of Benthic Habitats

At least 3 distinct major zones may be recognized readily on the lake-floor: littoral, sublittoral and profundal. A fourth, the "abyssal" is considered by some to occur in the deepest lakes, beginning at approximately 600 m. (Carpenter, 1928; p. 180) and extending to the greatest depth. The relative extent of these zones in different lakes varies to a marked degree, and indeed in some lakes the whole bottom may be said to be within the littoral. Muttkowski (1918, pp. 378-381) gave a classification of both limnetic (pelagic) and benthic habitats and proposed several terms to replace those which, perhaps, had been borrowed from oceanography. As yet his proposals have not been generally adopted by limnologists. Some other authors use such descriptive terms as "shallow water zone," "intermediate zone," and "deep-water zone." Lundbeck (1926, p. 116) summarizes the classification of bottom habitats by various authors, accepts the three terms, littoral, sublittoral and profundal and compares the extent of these zones as variously defined by himself and by others. It is not the purpose of the present writer to attempt to decide the relative merits of these various systems of terminology. But until some definite agreement has been reached by limnologists in general with respect to them it may perhaps be as well to use the older and more generally accepted ones, if only in the interest of uniformity.

In this paper, the littoral zone of the bottom is understood to mean that region lying between the shoreline and, approximately, the lakeward limit of aquatic vegetation. The profundal zone extends from the greatest depth (in lakes here considered) up the slope toward shore to a point somewhat above that corresponding to the average upper limit of the hypolimnion. The sublittoral lies between these two.

In Douglas Lake the littoral zone would thus extend from the shore line to a depth of about 9 m., the sublittoral, 9-15 m., while the profundal includes all the bottom below the latter depth, 15-28 m. In Third Sister Lake the three major benthic zones have the following approximate extent: littoral, 0-3 m.; sublittoral, 3-10 m.; profundal, 10-18 m. Kirkville Green Lake has a narrow littoral zone which probably in no place extends below 3 m.; the sublittoral may reach to 6 or 8 m.; and the profundal (8-61 m.) would thus cover by far the larger portion of the bottom.

BOTTOM DEPOSITS

The bottom within the littoral zone in Douglas Lake is predominantly sandy. The submerged terrace which occurs around practically the whole shore is built almost entirely of sand, except where headlands jut into the lake as at Grapevine, Stony and Roberts Points and at Fairy Island. At these places the shelf is composed of gravel, cobblestones, and small boulders mixed with the sand. The principal vegetation region of the lake is in the western end, where, protected from strong wave action induced by prevailing northwesterly winds, emergent and submerged vegetation has reached its richest development. Except for a fairly extensive area of *Scirpus validus* Vahl. on Big Shoal and some other, minor, exceptions in small coves, vegetation in the eastern part of the lake is confined to the usually steep slopes extending downward from the edge of the submerged terrace. Only in rare instances has the writer brought up bottom samples from below 9 m. which contained any living plants. The whole central portion of the lake, bordered by Grapevine Point, Sedge Point and Stony Point depressions on the east and by Roberts Point depression and Fairy Island on the west, is shallow and lies partly within the littoral and partly within the sublittoral (Fig. 3). Much of this region supports a considerable growth of submerged vegetation and over the entire area bottom deposits are sandy mud. Both in this central region of the lake and within the sublittoral on the slopes of the depressions there are evidences of a weakly developed shell zone.

The sublittoral is truly a transition zone in Douglas Lake. Bottom deposits within it are characterized by a change from the sand of the littoral to the mud of the profundal. In North Fish-Tail Bay, where the slope is not so abrupt as in many parts of the lake, the change from a distinctly sandy bottom to a distinctly muddy one takes place between depths of 9 and 11 m. Repeated sampling in series, usually beginning where the water was about 5 meters deep, and extending to the center of the bay, showed clean sand up to depths of 8 or 8.5 m.; the first noticeable amounts of mud at 9 m.; muddy sand at 9.5-10 m.; sandy mud, 10-11 m., and beyond that depth no noticeable amounts of sand. In small, more protected coves, mud extends to shallower depths and there vegetation attains its best development.

Around the edges of all major depressions, sand persists in considerable amounts down to a depth of 15 m., and along the eastern side of Sedge Point depression, the bottom consists of muddy sand as far down as 21 m. This may be due to carrying of sand into the depression by undertow currents off of the Big Shoal. In Maple Point depression, located just west of Fairy Island, the author has never found anything but clean or slightly muddy sand on the bottom.

The lake floor of the profundal zone, which in Douglas Lake occurs entirely within the major depressions, is carpeted with a thick layer of fine,

black ooze. Superficially, this mud appears to be homogeneous in texture and of a uniform character in each of the depressions. Welch (1927, p. 430-431) points out the fact of depression individuality in regard to physical-chemical conditions of the water, and early in the present work certain well marked differences, principally quantitative, were noticed in the bottom fauna of the six deeper depressions. The question as to whether this depression individuality also extended to bottom deposits naturally arose. Accordingly, two series of samples, all of equal volume, were collected near the center of each area of deeper water and examined. In collecting mud, the dredge was allowed to settled through the watery upper layer into firmer mud beneath.

One series of samples was put through a set of 6 Tyler screens to determine amount and character of coarse parts of the mud. Table IV presents data obtained from examination of mud collected in South Fish-Tail depression. However, mud from each of the other depressions, from the

TABLE IV. *Physical examination of bottom mud. Samples taken from the center of South Fish-Tail depression at 23 m. depth.*

Tyler Screen No.	Size Mesh mm.	% Passing through	Color and consistency of part remaining in screen
1	4.699	99	Black. Mostly plant materials. Leaves, etc.
2	2.362	98	Black. Plant and animal remains. Bits of leaves; plant fibers, fish scales and bones, etc.
3	1.168	90	Black. Mostly plant fibers.
4	0.417	85	Black. Fine debris of plant and animal origin.
5	0.208	80	Black. Very finely divided organic matter of plant and animal origin.
6	0.147	70	Black. Extremely finely divided organic matter of animal and plant origin, mixed with finely divided inorganic "dust."

deeper water of North Fish-Tail Bay, and from Grapevine Channel—the narrow neck of deep water between Grapevine Point and Big Shoal—was also examined in the same manner. The relative proportions of coarser debris (that part retained by the complete set of screens) and dust fine detritus (that part passing through the finest screen) remained, however, fairly constant. The percentage passing through the finest screen varied from 68.7 in Sedge Point depression to 74.4 in Fairy Island depression, an extreme variation of only 5.7 per cent. Table IV shows that 10 per cent of the mud is relatively coarse and consists of bits of both plant and animal remains, 20 per cent is finely divided organic material but still not fine enough to pass through a screen with 100 meshes per inch (0.147 mm.), while the remaining 70 per cent constitutes the dust fine detritus referred to above. Practically all inorganic material in the profundal mud is contained in this extremely finely divided matter.

The second series of samples was used for the purpose of determining (1) weight per unit volume of wet mud, dry mud, residue after ignition, and loss on ignition; and (2) percentage of water and solid matter in the wet mud, and percentage of volatile and non-volatile matter in the dry mud. Table V gives details of this analysis. The samples of wet mud were placed in previously weighed, wide mouth jars. These were tightly closed in the field. Later, in the laboratory, the wet mud was weighed on heavy analytical balances and then dried in an electric oven at 60°C. for 96 hours. The dry mud was then cooled in a dessicator; weighed on a delicate analytical balance to the nearest whole milligram; ashed in a fused silica crucible over a gas blast lamp for 40-50 hours; again cooled in a dessicator; and finally the ash (residue after ignition) weighed on an analytical balance. The difference between weight of the dry mud and weight of the residue after ignition (*i.e.,* loss on ignition) represents approximately the weight of the dry volatile matter, and the weight of the ash represents non-volatile matter present in the dry mud. Table V also gives data for Third Sister Lake which will be discussed later.

It is evident that bottom deposits are not uniform in all depressions of Douglas Lake. The muds from South Fish-Tail and Fairy Island depressions are most alike and this similarity may be due to the protected position of the former and the large size of the latter, since both factors would tend to minimize the amounts of heavier inorganic matter carried into the center of the depressions. Differences of bottom deposits in various depressions in terms of weight of dry mud, ash, and loss on ignition, and in ratios between water and solids and between volatile and non-volatile matter are not great when mud from a given depression is compared with that most like it. But if, on the other hand, the greatest differences be sought, as for instance, those between the mud from South Fish-Tail depression and that from Stony Point depression, then some appearance of depression individuality is evident. This is more significant when it is pointed out that the samples analyzed were portions of thoroughly mixed larger samples composed of 5 dredge-fulls of mud taken from widely scattered points within the deepest water area.

The floor of Third Sister Lake is predominantly muddy. There is a short stretch of about 100 m. along the southeastern shore where the bottom is firm and contains some sand and on the northern side there is a shorter reach of firm bottom along shore. Aside from these two regions, bottom deposits are largely mud from shore to lake center. Differences between the bottom within the littoral and the profundal zones, which are so pronounced in Douglas Lake, are not nearly so great in Third Sister. In the latter, the plant zone is largely restricted to the area above the 2 m. contour with scattered patches of vegetation extending to a depth of 3 m. There is a weakly developed shell zone in isolated spots within the sublittoral, located most often at a depth of 5-6 m., and also within this zone bottom deposits lose all littoral characters

and change to a stratified mud which covers all the bottom below the 10 m. contour.

The stratification of bottom deposits within the profundal zone of Third Sister Lake is one of the most apparent of their characteristics. If a sample of this bottom mud is carefully removed from the dredge it will retain, very nearly, the shape of the dredge opening and will show, on its edges, clearly defined alternate layers of a soft, black, organic detritus and a much firmer grayish clay. The thickness of these two kinds of layers was measured in many samples, and, although there was much variation, the average thickness may be stated as about 1-2 cm. for the clay strata and 0.5-1 cm. for the ooze layers. The dredge ordinarily penetrated to a depth of only about 8-10 cm. in the relatively firm mud and no increase in density of the lower layers could be discovered in this slight thickness.

The clay strata of this bottom mud are so firm and sticky that they can, with a little care, be separated from the ooze layers and in this way it is possible to get practically pure samples of each layer. The last 3 lines of Table V show results of analyses of (1) bottom mud as it occurs in the lake (1a), *i.e.* alternate layers of clay and ooze, which for purposes of analysis were thoroughly mixed together; (2) the organic detritus layers (1b), and (3) the clay strata (1c). The results from the mixed mud show a very surprising approximation to the average of the values for the two layers taken separately. While it is true that the two kinds of material were present in about equal proportions, by volume, this striking similarity of the mixture to the mean of its two parts is almost certainly accidental in the case of these data. Many analyses would be needed before any such close agreement could be shown to be constant or even usual. However, as in the instance of the Douglas Lake mud, these samples were parts of larger, composite samples which were collected from various points within the 18 m. contour and hence the data have a reasonable validity.

Reference to Table V will show that the black detritus layers are somewhat like the profundal bottom deposits of Douglas Lake, the percentage of organic matter being most nearly like that in the South Fish-Tail depression mud and the ratio between solid matter and water in wet mud at least comparable to the Stony Point depression mud. The clay strata, however, are entirely unlike anything found in Douglas Lake and this difference is so pronounced that mud taken as it occurs on the lake floor (1a) is also greatly different than any profundal mud in Douglas Lake.

Evidence from several sources seems to indicate that the clay strata are deposited largely during the spring and that the black detritus layers are laid down during the rest of the year. The explanation is two-fold. Deposition of black ooze probably goes on throughout the year, but is perhaps greatest during summer and autumn. Repeated observations at all times of the year and extending over several years have shown that the small intermittent

stream entering the lake carries very heavy loads of clay and silt. Some material of this nature is thus brought into the lake from the time ice disappears in the spring until it forms again in early winter. This is reflected in the detritus layers which, themselves, are made up of mud that is heavier than the heaviest from Douglas Lake. But in summer and autumn the fields are covered with vegetation, and this fact, possibly together with others, greatly reduces the amount of clay brought into the lake by the creek during these seasons. On the other hand, the thaws of late winter and the heavy rains of spring find the fields bare of vegetation and it is then that relatively very large amounts of clay are carried into the lake.

Direct evidence, from field observations, shows that the ice is frequently coated with a layer of clay, deposited when the surface is flooded by the first thaws of late winter. This load is, of course, dropped into the lake when the ice melts. Furthermore during the spring the creek is seldom dry, its waters are very muddy, and the whole body of the lake is frequently so turbid for days at a time that Secchi's disc cannot be seen below 1 m., whereas the disc reading during other seasons of the year averages about 5 m.

Bottom deposits of other lakes considered in this investigation received only casual study, but the following observations seem worthy of inclusion. The most characteristic thing about the shallow water bottoms in Kirkville Green Lake is the extensive deposits of marl. In the deep water (below 15 m.) the bottom is composed of a fine, black ooze which has a distinctly greasy feeling and a very offensive odor.

SUPERIMPOSED WATER

The relation of superimposed water to profundal benthic habitat is an important one in lakes of the type studied; *i.e.*, those which typically show thermal-chemical stratification in summer. In any attempt to determine extent of the various benthic zones, the physical-chemical conditions, as imposed by thermal stratification of the water are perhaps the most important factors. Certainly this is true so far as the profundal region is concerned. Plant growth is a useful and significant criterion in the upper zones and bottom deposits must be considered at all levels, while depth, although influencing all the other factors, is not, of itself, a constant or dependable index, since the ratios between it and other factors vary greatly in different waters.

In the lakes on which this study has been conducted, the typical profundal benthic habitat is understood to mean, in general, that region of the lake floor which lies beneath the hypolimnion and is therefore subjected to continuously low temperatures; little or no light; a pH which frequently falls below neutrality; considerable amounts of free carbon dioxide; a complete absence of dissolved oxygen during part of the summer and often during late winter; and, at least in some lakes, accumulations of methane, hydrogen sulfide, and perhaps other decomposition gases.

The upper limit of the hypolimnion in Douglas Lake varies somewhat with the time of year and from season to season; and it frequently is different in each depression. However, if all the data available on all the depressions are considered, the average for the whole lake over a period of years is near 15 m. Tables 6-11 give, in summarized form, data on the 6 major depressions of this lake for the years 1923, 1926, 1927 and 1928. It will be seen from these tables that, excepting Stony Point depression which is the shallowest of the six and which frequently does not stratify thermally, the bottom beneath the hypolimnion is a region subjected to typical profundal conditions.

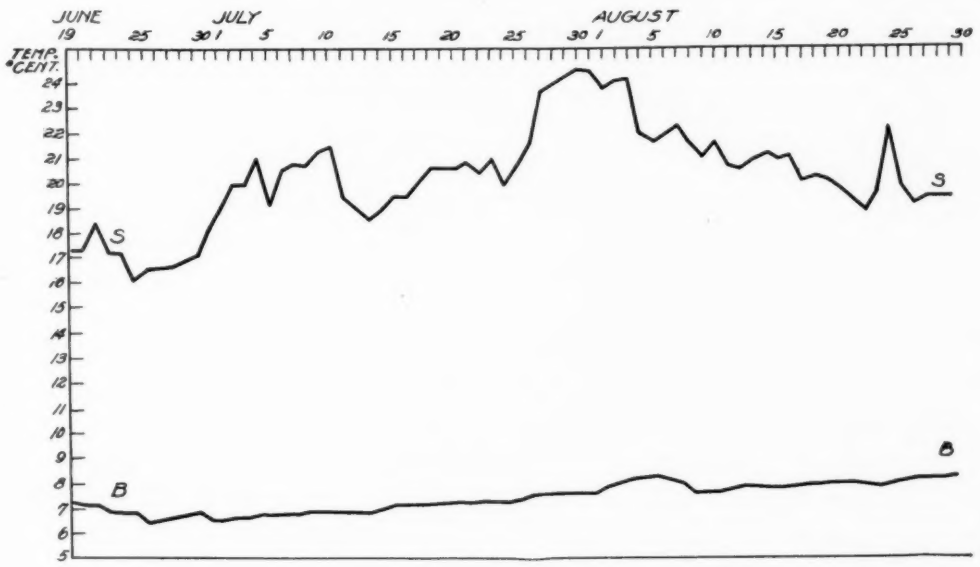
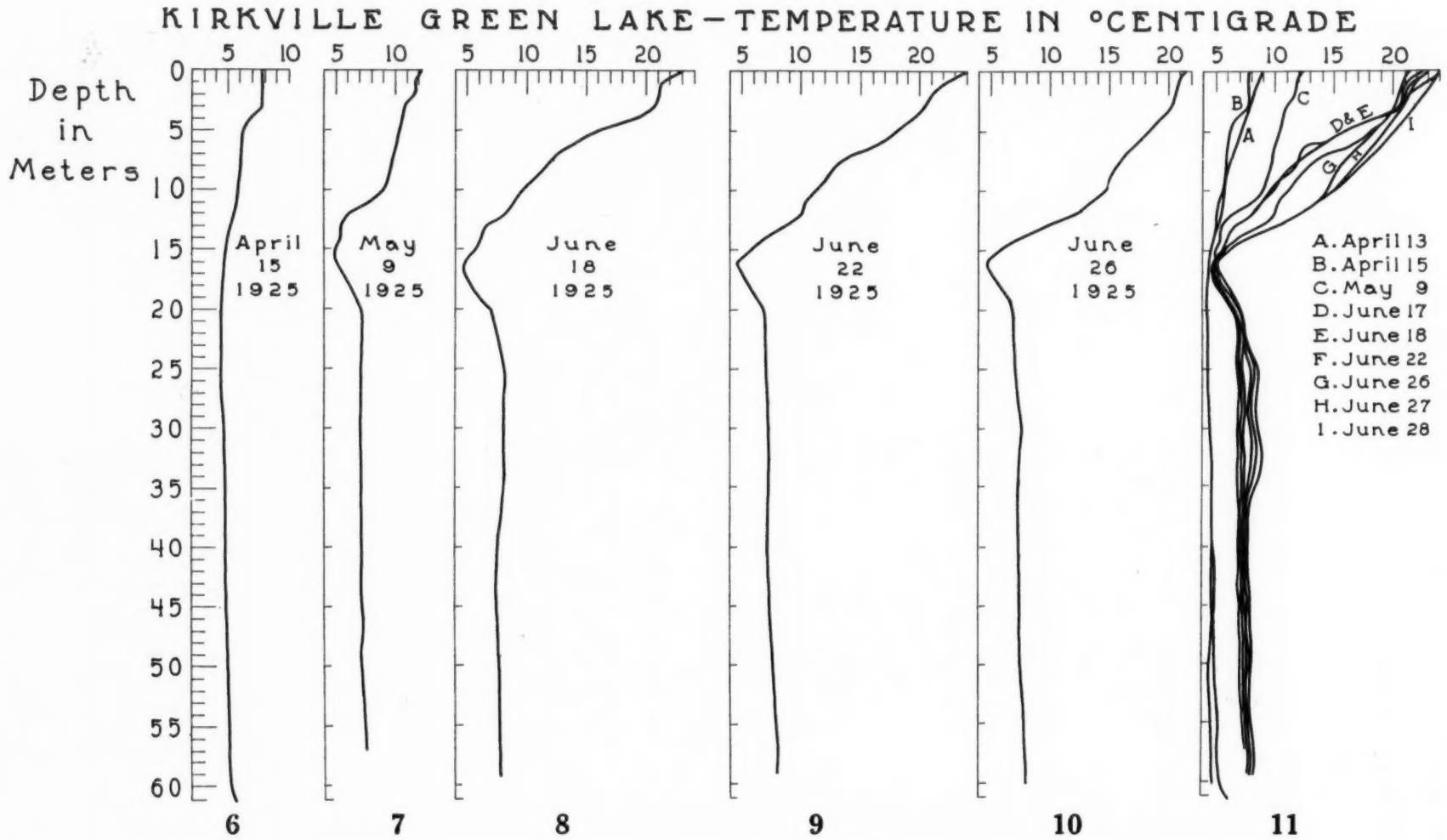


FIGURE 5

Figure 5 gives daily surface and bottom temperatures, taken in South Fish-Tail depression during 1926. The increase in temperature of the bottom mud during the summer is slight. It may be pointed out that the increase is continuous and steady and that it lags slightly behind a similar rise in temperature of the bottom water. The figure shows clearly the comparative uniformity of low temperatures at the bottom.

The deeper waters of Third Sister Lake show a seasonal cycle of physical-chemical phenomena which approaches even more closely that of the theoretically typical stagnation zone. A summary of the data concerning temperature, pH, dissolved oxygen and free carbon dioxide, at the surface, upper limit of the hypolimnion, and at the bottom, on selected dates in 1923, and between October, 1926, and February, 1929, is given in Table XII. Thermal stratification of this lake probably begins much earlier in the spring than in Douglas



FIGS. 6-11. Temperature distribution in Kirkville Green Lake on selected dates in 1925. Depths in meters and temperatures in degrees Centigrade.

Lake and the few available fall records for the latter (Tables VI and XI; October 20, 1928) indicate that the period of summer stagnation also persists longer in the fall. Here again, occurs a typical profundal benthic habitat and, coincident with a longer period of stagnation, physical-chemical conditions on the lake floor beneath the hypolimnion are even more severe than in Douglas Lake. The shape of the basins, the character and distribution of the plants, the nature of the bottom deposits and of the waters indicate that Douglas and Third Sister Lakes belong to the type often designated as *eutrophic*.

Kirkville Green Lake is roughly flask-shaped. The body of the lake and one side of the neck are surrounded by high, wooded bluffs and it is thus unusually well protected from wind disturbance. Indeed, even when relatively high winds are blowing, its surface is scarcely more than rippled. This fact, together with the steep slope of its floor, the black, greasy character and the foul odor of its deeper mud deposits (over which it was at times impossible to work more than a few minutes without becoming nauseated) and the peculiarities of its temperature distribution (Figs. 6-11; Table XIII) leads the author to suspect that the lake may have incomplete spring and fall overturns. It may even be possible that complete circulation is wholly absent. If this be true, stagnation would become particularly effective and, not being relieved by the usual semi-annual circulation periods, would produce a profundal benthic habitat of the most severe sort.

Some features of Kirkville Green Lake, such as its steeply sloping bottom, its clear water, and the slight development of its littoral zone, would seem to place it in the *oligothropic* class. Other characters, just discussed, perhaps indicate that its profundal zone is more nearly like that of the *dystrophic* lakes.

PROFUNDAL BOTTOM FAUNA

General Features

On a qualitative basis, the bottom samples collected must be considered in two groups. In one group are those which were taken in sampling series extending from deep to shallow water. Usually such series were begun in the deepest water and, with samples taken at frequent intervals, extended as far up the slope as any profundal bottom animals were found. Sometimes such series were continued into shallower water, and in some instances a certain amount of sampling was done wholly in moderate depths. The animals secured in these samples, obviously, would not form a homogeneous group, but, instead, changed with the decrease in depth.

The bottom samples taken entirely within the true profundal zone form the other natural group. Here the organisms secured constitute a somewhat homogeneous and very distinct ecological association. This fauna is qualitatively poor but quantitatively very rich. It makes up the typical profundal bottom fauna and includes representatives of the genera Corethra, Chi-

ronomus, and Protenthes, among the Insecta; Limnodrilus among the Oligochaeta; Pisidium and Musculium among the Mollusca; and Hydromermis from the Nematoda. In the following section this last group will be considered first.

These types, and particularly representatives of the genera Corethra, Chironomus, and Protenthes and of the family *Tubificidae*, constitute a truly typical, profundal, benthic fauna in the lakes studied. This fact is clearly shown by Tables XIV-XVII, which give the seasonal variations of the bottom animals below 21 m. in South Fish-Tail, Sedge Point, Fairy Island, and Roberts Point depressions of Douglas Lake. Table XX gives similar data on bottom fauna below the 18 m. contour in Third Sister Lake. It will be seen that in each of these tables the column headed "all others" is, with certain exceptions to be discussed later, a record of an almost complete absence of any other animals. The fact that these animals occur in the sublittoral of both Douglas and Third Sister Lakes, as well as in the profundal, and the additional fact that some of them were found in the littoral zone in certain other lakes, do not render unsound their classification as profundal. They are here considered as constituting the typical profundal benthic fauna of the lakes studied, because they are the only benthic animals typically found within the true profundal zone during the time when the environmental factors which make it a profundal zone are at their maximum intensities.

ANNOTATED LIST

Notes included within quotation marks are quoted from comments written on the lists of identifications by the authorities who identified the specimens.

Typical Profundal Fauna

INSECTA

1. *Corethra punctipennis* Say. Professor Johannsen, in his list of identifications gave Chaoborus as the name of the genus and Corethra as a synonym. The author, however, prefers to use the better known name, Corethra, for reasons which need not be discussed here.

Larvae one of three most abundant organisms within true profundal zone; in all depressions of Douglas Lake, usually in all depressions at same time; never entirely absent from profundal zone in Third Sister Lake, at times in very large numbers; sometimes many larvae in water over this zone; frequently limited numbers in sublittoral zone of Douglas and Third Sister Lakes; not taken in littoral zone except when washed ashore by heavy on-shore night winds. Never found in Kirkville Green Lake. Only one species.

2. *Chironomus plumosus* L. Common within profundal, Douglas Lake; 12-28 m. Rare in Third Sister Lake; taken only at 10-12 m. Larvae *C.*

plumosus and *C. plumosus* var. *ferrugineovittatus* constituted greater part chironomid fauna within profundal zone in Douglas Lake.

3. *Chironomus plumosus* var. *ferrugineovittatus* Zett. Douglas Lake; slightly less numerous than *C. plumosus* in profundal zone; in all depressions and in deeper water, North Fish-Tail Bay. Not known from other lakes studied.

4. *Chironomus utahensis* Mall. Third Sister Lake, 8-18 m.; most common below 10 m. Larvae of this species and of *C. fasciventris* constituted practically all chironomids within profundal zone in Third Sister Lake. Not known from other lakes investigated.

5. *Chironomus fasciventris* Mall. Taken only in Third Sister Lake; 6-18 m., most numerous 6-12 m.; apparently outnumbered by *C. utahensis* approximately 2:1 within entire profundal zone.

6. *Protenthes culiciformis* L. Larvae in all depressions, Douglas Lake; 10-28 m.; most common in upper profundal and lower sublittoral. Larvae 3-18 m. in Third Sister Lake.

OLIGOCHAETA

7. *Limnodrilus hoffmeisteri* Clap. Douglas Lake, all depressions; 10-28 m. Third Sister Lake, 3-18 m.

8. *Limnodrilus claparedianus* Ratzel. Douglas Lake, profundal zone; all depressions. Third Sister Lake, 3-18 m.

SPHAERIIDAE

9. *Pisidium compressum* Prime. Douglas Lake, Grapevine Point depression, 24 m.; Roberts Point depression, 17-22 m.; South Fish-Tail depression, 13-23 m.; Grapevine Channel, 11-15 m.; Stony Point depression, 18 m.; North Fish-Tail Bay, 10.5 m.; mid-way between Roberts Point and Fairy Island depressions, 16-17 m. Third Sister Lake, 4-8 m. Most common representative of *Sphaeriidae* within profundal zone, Douglas Lake; never in more than limited numbers.

10. *Pisidium* sp. "Immature, somewhat like *splendidulum* St." Grapevine Channel, 17 m.; South Fish-Tail depression, 18 m., Douglas Lake.

11. *Musculium rosaceum* Prime. Stony Point depression, 17-19 m.; North Fish-Tail Bay, 10-12 m., Douglas Lake. Not common.

12. *Musculium truncatum* Linsley. "Apparently." A few immature specimens, Roberts Point depression, Douglas Lake, 21 m.

NEMATODA

13. *Hydromermis* sp. Douglas Lake; all depressions; Grapevine Channel; deeper water, North Fish-Tail Bay; 11-28 m.; most abundant in Roberts Point, Stony Point and Grapevine Point depressions. Occasionally in Third Sister Lake; 8-18 m.

Other Bottom Animals

The forms listed below are those taken in sampling series extending from deep to shallow water, exclusive of the typical profundal species. Included also are those forms taken in samples wholly from shallow water.

HYDRACARINA

1. *Arrhenurus cardiacus* Mar. Third Sister Lake, 6-12 m.
2. *Arrhenurus rectangularis* Mar. Third Sister Lake, 8-15 m.; 1 specimen.
3. *Diplodontus despiciens* (Müll.). Kirkville Green Lake, 1 m. Third Sister Lake, 6-12 m. "Cosmopolitan."
4. *Forelia liliacea* (Müll.). Douglas Lake, Roberts Point depression, 20 m. Few.
5. *Limnesia histrionica wolcotti* Piers. Third Sister Lake, 6-12 m. Few.
6. *Limnochares aquaticus* L. Kirkville Green Lake, 1 m. "Cosmopolitan."
7. *Midcopsus orbicularis* (Müll.). Douglas Lake, Roberts Point depression, 20 m. "Cosmopolitan species."
8. *Neumania semicirularis* Mar. A "common form." Douglas Lake. Roberts Point depression, 20 m. Third Sister Lake, 8-15 m.
9. *Piona interrupta* Mar. Kirkville Green Lake, 3 m.; Third Sister Lake. 4-18 m.

In Third Sister Lake, this is by far the most abundant hydrachnid, outnumbering all others combined. Writing of this species, Dr. Marshall states: "Known from Canadian and Wisconsin deeper waters. Male, heretofore unknown, occurs in the Third Sister Lake collections."

10. *Piona rotunda* (Kram.). Common in Third Sister Lake; 6-18 m.; Second only to *P. interrupta* in abundance.
11. *Unionicola aculeata* (Koen.) "nov. var." Third Sister Lake, 8-18 m.

Concerning this species Dr. Marshall writes: "Found for the first time in northern Wisconsin, 1928, where it was quite abundant." It was the third most numerous mite in Third Sister Lake collections.

12. *Unionicola crassipes* (Müll.). "Cosmopolitan." Third Sister Lake; 6-15 m. Fairly common.
13. *Unionicola pectinatas* (Wol.). Third Sister Lake; 8 m.

INSECTA

1. *Chironomus cagugae* Joh. Douglas Lake, Grapevine Channel, 12-15 m.; Roberts Point depression, 15-18 m.; South Fish-Tail depression, 15-17 m. Adults frequently resting on surface of water over edge of South Fish-Tail depression, both in early morning and late evening; also common on dock posts at Biological Station.

2. *Chironomus decorus* Joh. 1 specimen, reared from pupa taken from surface of water over Roberts Point depression, Douglas Lake.

3. *Chironomus devinctus* Say. South Fish-Tail depression, Douglas Lake, 15 m.

4. *Chironomus stylifera* Joh. Third Sister Lake, 6-10 m.

5. *Chironomus viridicollis* Vaw. Douglas Lake, South Fish-Tail depression, 12 m.

6. *Johannsenomyia sp.* Larvae relatively common in samples from shallower depths; occasionally in deep water: Roberts Point depression, 21 m.; South Fish-Tail depression, 20 m. Most abundant in North Fish-Tail Bay, Douglas Lake, 10-11 m. Commonly present, Third Sister Lake, 3-8 m.; Occasionally abundant, 7-8 m.

7. *Tanypus basalis* Walley. Douglas Lake; Grapevine Channel, 15-17 m.; few; South Fish-Tail Bay, 13 m., rare; 22 m., 1 specimen.

CRUSTACEA

Ostracoda. Ostracods occasionally in Douglas Lake bottom samples; specimens quite small; few, 13-23 m. Sometimes abundant Grapevine Channel, 12 m. Not common in Third Sister Lake, 3-18 m. Species not determined.

GASTROPODA

1. *Amnicola limosa* (Say). Douglas Lake, Roberts Point depression, 18 m.; North Fish-Tail Bay, 10-12 m.; Stony Point depression, 17-19 m.

2. *Campeloma decisum* (Say). Douglas Lake, Stony Point depression, 13-18 m.; South Fish-Tail depression, 12-13 m.; Roberts Point depression, 10-13 m.; Grapevine Point depression, 10-15 m.; North Fish-Tail Bay, 10-12 m. Third Sister Lake, 3-5 m.

3. *Campeloma rufum* (Haldeman). Stony Point depression, Douglas Lake, 15-18 m.

4. *Goniobasis depygis* Say, "var." Kirkville Green Lake, common around shore to depths of 3 m.

5. *Physa heterostropha* Say. Kirkville Green Lake, east and south of outlet, 1 m. North Bay, most of shore line; few.

6. *Planorbis altissimus* Baker. Douglas Lake, Roberts Point depression, 10-12 m.

7. *Planorbis antrosus striatus* Baker. Kirkville Green Lake, 1-5 m.

8. *Planorbis parvus* Say. Kirkville Green Lake, 1-2 m. Douglas Lake, North Fish-Tail Bay, 4-10 m.

9. *Succinea retusa* Lea. Kirkville Green Lake, near outlet. North Bay, 0-2 m., few.

10. *Valvata sincera* Say. Third Sister Lake, fairly common, 3-7 m., especially along southern and western shores.

11. *Valvata tricarinata* Say. Most frequently collected gastropod in Douglas Lake 10-19 m. All depressions and in nearly all samples from inter-depression regions. Frequent in North Fish-Tail Bay, 4-11 m.

12. *Valvata tricarinata perconfusa* Walker. Kirkville Green Lake along west shore, 1 m. Douglas Lake, North Fish-Tail Bay, 10-11 m.

13. *Valvata tricarinata simplex* Gould. Kirkville Green Lake along west shore, few, 0-2 m.

PELECYPODA

1. *Anodonta marginata* Say. A few on upper slopes, South Fish-Tail, Roberts Point and Stony Point depressions, Douglas Lake, 10-18 m.

2. *Lampsilis luteola* (Lamarck). Occasionally in Douglas Lake bottom samples. South Fish-Tail depression, 11 m.; North Fish-Tail Bay, 10 m.; Roberts Point depression, 12 m.; Grapevine Point depression, 10 m.

3. *Pisidium compressum* Prime, "(?), quite peculiar, a form of *compressum*, or near." Douglas Lake, Grapevine Channel, 11-15 m.

4. *Pisidium imbecille* St. Douglas Lake, North Fish-Tail Bay, 4-10 m.

5. *Pisidium variabile* Prime. "A form." Douglas Lake, North Fish-Tail Bay, 10-12 m.

6. *Pisidium sp.* "Apparently an undescribed species of the *compressum* group." Third Sister Lake, 3-8 m.; rather frequent.

7. *Pisidium sp.* "Apparently near *concinnulum* St." Both immature and mature specimens. Third Sister Lake, 4-8 m.

8. *Pisidium sp.* Stony Point depression, Douglas Lake, 18 m.; several very young specimens.

9. *Sphaerium acuminatum* (Prime). Douglas Lake, Roberts Point depression, 10-15 m.; Grapevine Channel, 11-15 m.

10. *Sphaerium nov. sp.* "Near *sulcatum* (Lam.), a new species, not published; from many places in Michigan, etc." Douglas Lake, North Fish-Tail Bay, 10-12 m.; Roberts Point depression, 10-14 m.

HIRUDINEA

No leeches were taken in bottom samples but several specimens representing the following species, were found in wire-netting cages which had been lowered to the bottom of Third Sister Lake during certain lake-bottom experiments.

1. *Erpobdella punctata* Leidy.
2. *Glossiphonia complanata* Linn.

NEMATODA

1. *Dorylaimus nov. sp.* (?) Bottom samples, Grapevine Channel, Douglas Lake: 10-15 m.; common, sometimes abundant, maximum number per sq. m. 770. Occasionally elsewhere in lake: South Fish-Tail Bay, 10-12 m.; North

Fish-Tail Bay, 10-12 m.; Stony Point depression, 12-18 m.; Roberts Point depression, 12-20 m.; Grapevine Point depression, 24 m.

Occasional Records

In addition to the animals referred to in the preceding lists, there are scattered records of many other species. None of them occurred with enough regularity, however, to warrant its inclusion in either of the lists. Among such animals were: larvae of *Sialis infumata;* several species of Ephemerida, an occasional trichopteran; a few Odonata nymphs; various naidids; and rarely a turbellarian, a fragment of a bryozoan, or of some fresh-water sponge.

COMPARISONS

Compared with that of other North American lakes in which bottom fauna has been studied, the profundal fauna of Douglas Lake resembles more closely, in a qualitative sense, that of Lake Mendota, Lake Wawasee, Lake Winnebago, Lake Simcoe and Lake Okoboji than does that of Third Sister Lake. Both Douglas and Third Sister Lakes have a profundal bottom fauna which is qualitatively different than that of Lake Nipigon, Green Lake (Wisconsin), Lake Ontario, the Finger Lakes of New York and Lake George, although in a general way they also have similarities. If differences of species are disregarded and only genera considered, then the profundal fauna of Third Sister Lake is practically the same as that in Douglas Lake and the lakes with which it is compared above.

In the fact that the most abundant chironomid larvae of its deep-water fauna belong to the *C. plumosus* group, Douglas Lake resembles Lake Pepin (Johnson and Munger, 1930) and the Illinois River and its connecting lakes from Chillicothe to Grafton (Richardson, 1928). Douglas Lake is, however, in no sense a polluted body of water and this fact indicates that *C. plumosus* is not always associated with pollution. This is especially interesting in the light of the great abundance of these larvae found in Lake Pepin and in the Illinois River. So far as its chironomids are concerned, Third Sister Lake, with *C. utahensis* and *C. fasciventris* forming the bulk of that group of profundal benthic animals, is unlike any other lake known to the author.

Corethra punctipennis and *Protenthes culiciformis* are present in considerable numbers in the bottom fauna of both Douglas and Third Sister Lakes and in this respect these lakes are like Lake Mendota, Lake Simcoe, and Lake Wawasee (and apparently Walnut Lake), but unlike Lake Nipigon, where neither of these species was taken, Lake Pepin, where only a single specimen of Corethra was found, and also unlike Lake George, Green Lake in Wisconsin, the Finger Lakes of New York and Lake Okoboji.

The two oligochaetes, *Limnodrilus hoffmeisteri* and *L. claparedianus* occur in both Douglas and Third Sister Lakes and here again it is interesting to note that markedly polluted reaches of the Illinois River are the only other local-

ities, of those here mentioned, from which these two species have been reported. It is true that specific determinations of the oligochaetes have not usually been obtained, and possible that if they had been, there would be more records of these two species in unpolluted waters of American lakes.

In general terms, the profundal benthic faunas of Douglas and of Third Sister Lakes are comparable with those in many European lakes. This is especially true of the "*plumosus-Corethra* type" common among the lakes of Northern Germany. The bottom faunas of certain Swiss lakes, for example St. Moritz and Le Leman, are quite unlike those found in any lake studied in this investigation.

Since the bottom fauna of the littoral and sublittoral zones did not come within the scope of the major problem considered in this paper, but was only considered incidentally when representatives were taken in bottom samples, no attempt will be made to compare this fauna in Douglas and Third Sister Lakes with that in other lakes. It may, however, be mentioned in passing that, so far as is known, the shallower water benthic fauna in the lakes studied by the writer has many points of similarity with that in Lake Mendota, Lake Wawasee and Oneida Lake.

SEASONAL VARIATION AND DEPTH DISTRIBUTION

VARIATIONS

The bottom fauna within the profundal zone, in the lakes studied, has shown clearly defined seasonal variations. Such changes have been of two sorts: (1) variations in species present, and (2) fluctuations in total numbers of the population. These phenomena will accordingly be considered under two headings, qualitative and quantitative.

Qualitative Seasonal Variations

One phase of the writer's investigations has been a study of any possible qualitative variations of bottom fauna within the true profundal zone. Physical-chemical determinations soon demonstrated that there were relatively large areas both on the bottom of Third Sister Lake and within the major depressions in Douglas Lake which constituted typical profundal habitats. In order, however, to have a region for study where the environmental conditions of the benthic habitat were at the maximum of their intensities, the area beneath the deepest water in both lakes was selected. Therefore, the collection of samples for this phase of the problem was restricted to the area within the 18 m. contour in Third Sister Lake, and the 21 m. contour in Douglas Lake for all depressions except Stony Point, where the 17 m. one was chosen.

Between October 20, 1926 and February 9, 1929, 163 samples were taken from below the 18 m. contour in Third Sister Lake. Table XX presents a summary of these data. It will be seen that each spring (in April) and each

fall (late in November or early in December) the area below 18 m. was invaded by organisms other than those which inhabited that region during the rest of the year. Such invasions are shown in the table in the next to the last column. Shortly after November 24, 1926, a layer of ice formed over the lake too thin to support a man's weight and yet thick enough to prevent the use of a boat. Hence no bottom samples were taken again until January 5, 1927, and as a result the appearance of the invaders was missed that fall. That such transitory bottom species occurred below 18 m. sometime in December of that year seems certain when it is pointed out that on November 24 there was a population of 23 animals per sq. m., in addition to the typical profundal species on the bottom between 16 and 17., (Table XXI). These bottom species which periodically invade the deepest water of this lake in the spring and fall are usually water mites, ostracods and, on rare occasions, specimens of Hydromermis.

Even within the typical profundal fauna there is a seasonal qualitative variation. A glance at the data in Table XX will show that Protenthes is by no means a constant inhabitant of the deeper zone and even the Chironomus larvae disappear at times. Thus it often happens that in the late fall the list of benthic inhabitants beneath the deepest water is reduced to two namely; Corethra larvae and the tubificids. At no time was a sample taken from this region of Third Sister Lake, however, when either of these two types was absent.

In Douglas Lake, practically all samples were taken during the months of June, July, and August. One result of this fact is an apparent absence of pronounced qualitative seasonal variation in the profundal zone. However, in view of the repeated autumnal and vernal qualitative changes in the bottom fauna within the profundal zone of Third Sister Lake, a special trip to Douglas Lake in late October seemed advisable in order to determine whether or not a similar change took place there. Accordingly, Douglas Lake was visited October 19-21, 1928, and data on the bottom fauna in South Fish-Tail and Roberts Point depressions were secured. The last line of Table XIV shows that a qualitative change had begun to appear beneath the deepest water in South Fish-Tail depression. Although only typical profundal species were as yet present in the deepest water a distinct change was evident within this group. The quantitative change was most striking, but it may also be pointed out that Protenthes larvae which had been absent from July 30 to August 12 were then present in appreciable numbers. The data for Roberts Point depression are, however, more convincing. In this depression there could be no doubt of the qualitative changes. A few ostracods were taken along with the typical profundal animals at 22 m. on August 6, and August 10, the number of ostracods had decreased slightly but a few Johannsenomyia larvæ were present. In the samples of October 20 there were found ostracods, hydrachnids, larvae of *Sialis infumata* and a few Dorylaimus.

A summary of all bottom samples within the deeper profundal zone in Douglas Lake is given in Tables XIV-XIX. These data are arranged according to depressions and show the results for the summers of 1923, 1926, 1927 and 1928. Examination of these tables demonstrates that the qualitative variation within the typical profundal fauna, which was evident in Third Sister Lake, also occurs in Douglas Lake. There are certain differences, however. In Douglas Lake not only do Chironomus and Protenthes disappear entirely beneath the deepest water at certain times but the tubificids also are frequently absent. This means that at times nothing but Corethra larvae or pupae are to be found in the mud at these depths. And although Corethra larvae never entirely disappeared in any depression of Douglas Lake, there were many occasions when they were almost completely absent.

Depression individuality in terms of qualitative differences of the bottom fauna is most clearly shown when the data for the depressions are arranged in two groups. In the larger group are South Fish-Tail, Fairy Island, Sedge Point and Roberts Point depressions. This group is typified by an almost complete absence of anything except Corethra, Chironomus, Protenthes, and *Tubificidae* from the deeper profundal zone during the summer months. The smaller group includes Stony Point and Grapevine Point depressions where the bottom fauna typically included several other species.

It will be seen that in Tables XVIII and XIX the column headed "All Others" contains records of a miscellaneous bottom population which occurred beneath the deepest water in Stony Point and Grapevine Point depressions. In the latter, the types most frequently found, in addition to the four given in the tables, were, Hydromermis, Dorylaimus, and Pisidium. In Stony Point depression, the list is considerably longer. In the "All Others" column of the table for that depression are included records for Hydromermis, Dorylaimus, Musculium, Pisidium, Amnicola, Campeloma, Valvata, and Ostracoda. Although *Sphaeriidae* occurred within the profundal zone in Douglas Lake, they were seldom taken below 21 m., and then only specimens of *Pisidium compressum* could be expected.

Quantitative Seasonal Variations

A clearer idea of the magnitude of quantitative faunal variations within the deeper profundal zone is to be had by a glance at Fig. 12, where number of organisms per sq. m. below the 18 m. contour in Third Sister Lake is shown graphically. The curves are plotted on a scale based on a numerical progression. This scheme was resorted to in order to allow plotting curves for all the organisms on one graph. It will be realized that the effect of this is to somewhat reduce the peaks of the lower curves and enormously reduce the Corethra and "totals" curves in proportion to the others.

In general, the most uniform in point of numbers present are the tubificids. If the more or less ephemeral, miscellaneous group, called "all others,"

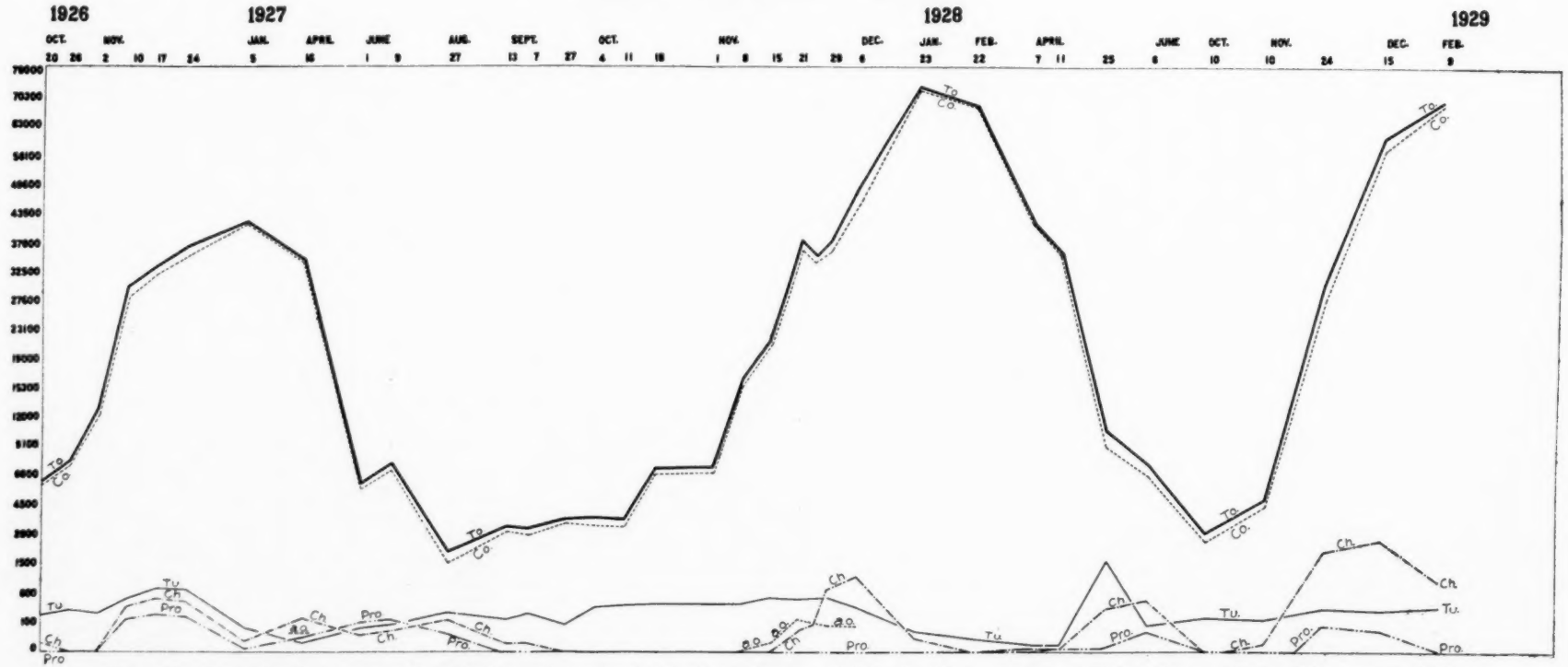


FIG. 12. Quantitative seasonal variation of profundal bottom fauna within the 18 m. contour, Third Sister Lake. The curves indicate number of individuals per square meter of total benthic fauna (To), Corethra larvae and pupae (Co), Chironomus larvae (Ch), Protenthes larvae (Pro), Tubificidae (Tu), and all others (a.o.).

(a. o. in the figure) is omitted from consideration, the lower curves all show a general similarity. Chironomus varied from complete absence on various dates to a maximum of 2400 per sq. m., on December 15, 1928. Protenthes larvae were absent for months at a time and their maximum (November 17, 1926) was only 200 per sq. m. The tubificids varied from a minimum of 23 to a maximum of 1550 per sq. m. of bottom. This maximum figure was obtained by the combination, and consequent averaging, of all samples from the 16, 17, and 18 m. depths on April 25, 1928. Studies on the depth distribution of these animals, as will be shown later, make it obvious that this accounts for the sudden increase in numbers shown in the curve. Thus at 18 m. on April 7, there were 23 tubificids per sq. m., on April 11 there were still 23, then, by the method mentioned above, the number jumped to 1550 on April 25 but had fallen off again on June 6 to 90 per sq. m. Quite evidently, the figure of 1550 for April 25 is very much too high for the 18 m. zone, although it does state the fact for the whole area of bottom below 16 m. If this false maximum be omitted, then the quantitative variation of the tubificids was from 23 to 734 per sq. m. The population of the miscellaneous group, "all others," reached a maximum of slightly more than 100 on November 21, 1927, and, on several other occasions, approached 90 individuals per sq. m. These animals are present within the profundal zone, as was pointed out before, only in the spring and autumn.

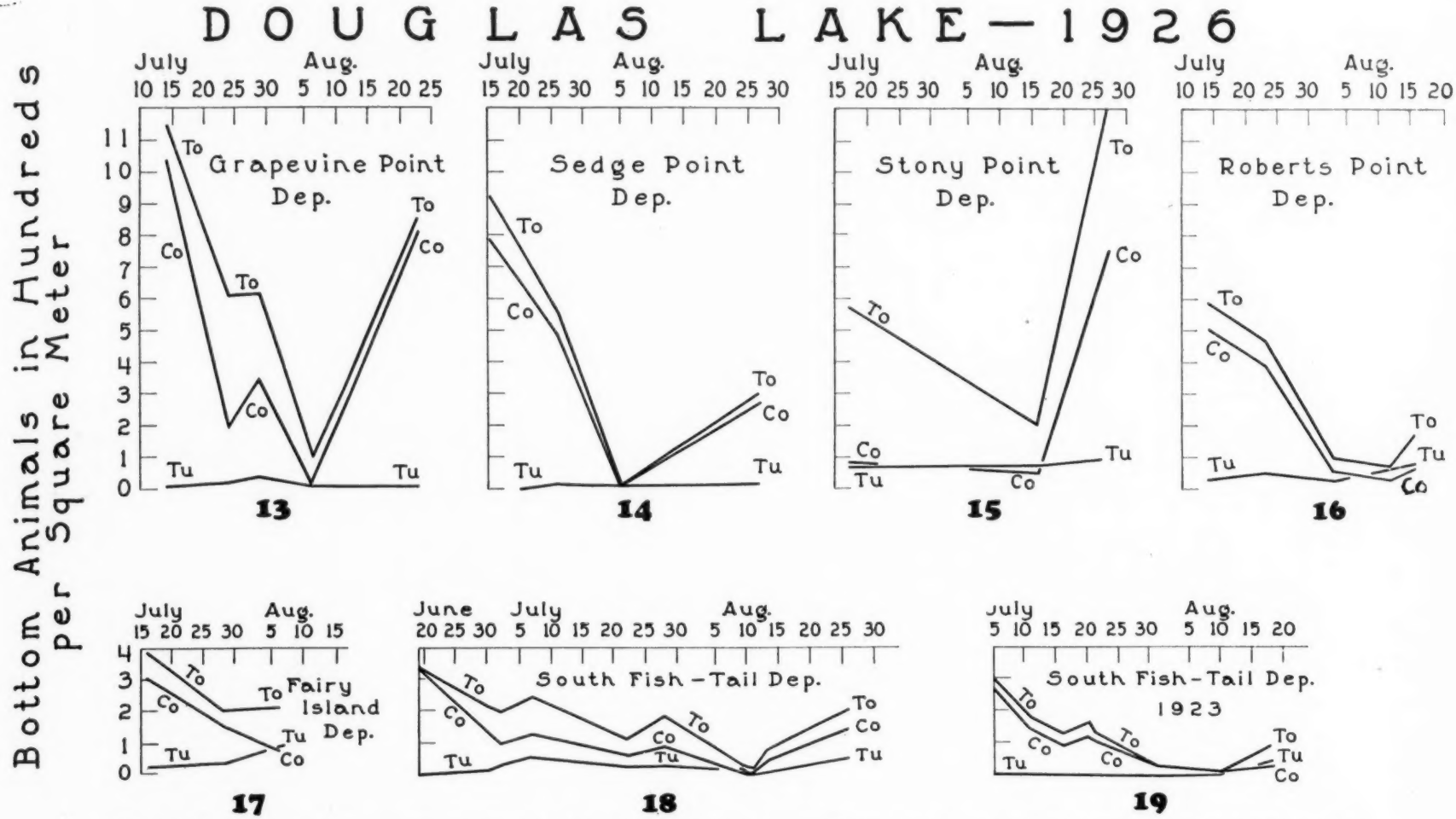
The curves for "Corethra" and the "total" attract attention in the figure. A glance is sufficient to show that the "total" curve is, in all its aspects, little more than a reflection of the Corethra curve. Corethra larvae dominate, numerically, the life of the profundal benthic habitat in Third Sister Lake in a striking way. During this investigation their numbers have never fallen below 1500 per sq. m. of bottom surface within the 18 m. contour, and they have reached a maximum of over 71,500 sq. m. The maximum found in Lake Mendota (Juday, 1922) of 33,800 per sq. m. seemed large indeed but the number in Third Sister Lake, per unit area, is more than double that figure.

The fluctuations of total profundal bottom population in the deepest region of Third Sister Lake showed a clearly defined periodicity. In late summer and early autumn, there was an annual minimum followed later in the fall by a very sudden and considerable increase in all species. This increase continued, so far as total population was concerned, until midwinter, when the annual maximum was attained. During the winters of 1926-7, 1927-8, and 1928-9, this maximum occurred in January and February. During March in each of these years, ice conditions were such as to prevent work on the lake but by early April, when the first spring observations were made, the benthic population in the center of the lake had materially declined. The decrease in numbers continued slowly throughout the spring and summer until the minimum was again reached in late August or early September.

The increase in autumn was not entirely simultaneous for all organisms concerned but was first evident in the Corethra larvae. This was followed by an augmentation of the population among the other bottom animals. That the autumnal increase is a sudden and pronounced one each year is attested by the data. Thus on October 26, 1926, the total population of the profundal bottom within the 18 m. contour was slightly more than 7,700 per sq. m. One week later the total per sq. m. of bottom within the same contour was a little more than 12,800, and again 8 days later the figure was approximately 29,700. In the autumn of 1927, the onset of the change was about one week later and the increase in total number of animals per sq. m. was as follows: November 1, 7,360; November 8, 16,200; November 15, 21,700; November 21, 37,700. Again in 1928, a similar change took place between November 10 and 24 with an increase from 4,900 on the former date to 30,600 on the latter or an increase of 25,700 individuals per sq. m. in 14 days.

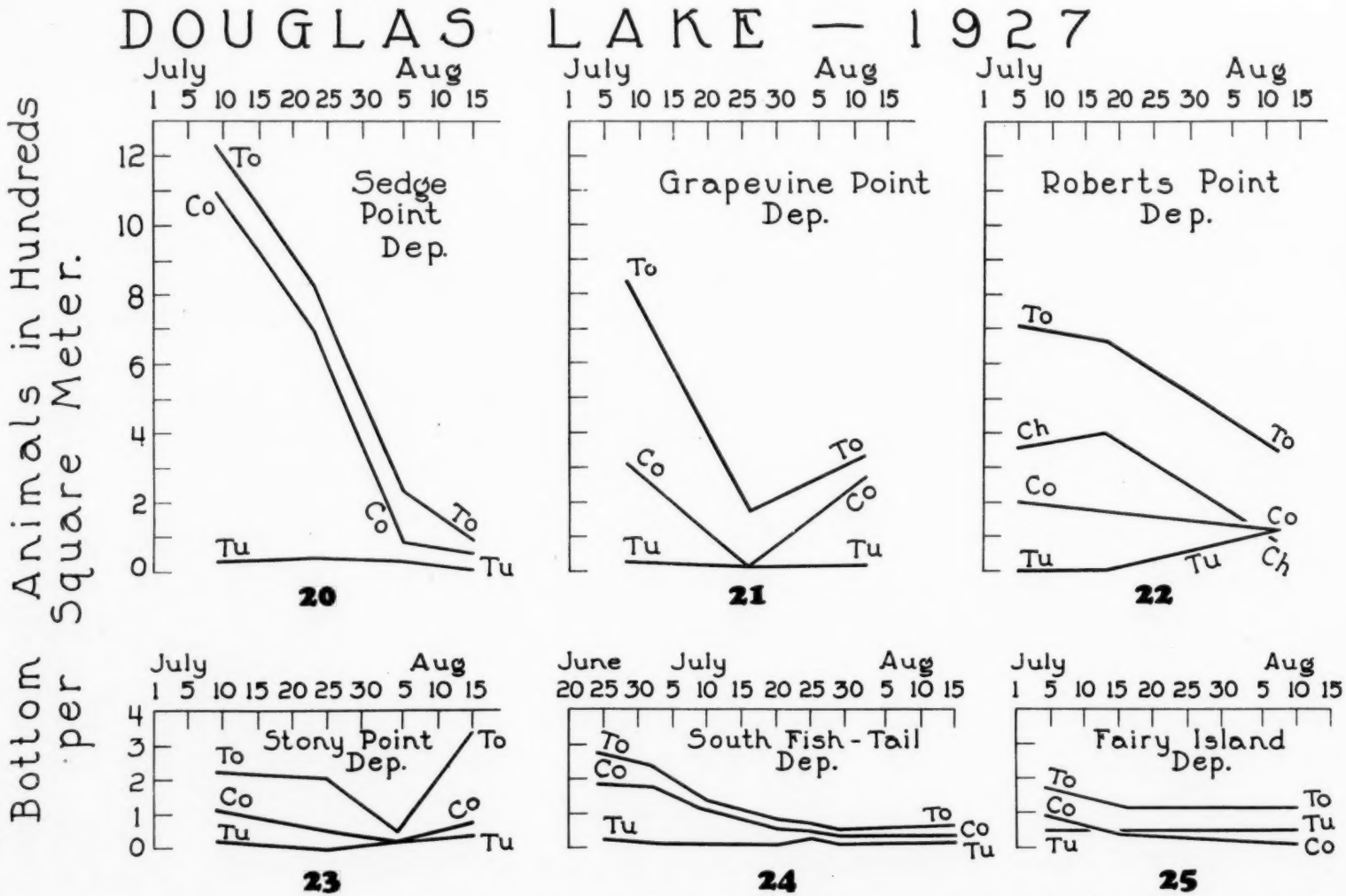
It is true, as has been pointed out before, that these fluctuations of total number of animals per unit area in the deeper profundal is very largely determined by changes in numbers of Corethra larvae. However, the autumnal increase is often just as abrupt in the case of other types, although not of such a magnitude. An instance of this kind is shown by the data for November 2 and 10, 1926. On the first date, the bottom population per sq. m. was found to consist of 12,665 Corethra larvae, and 171 tubificids. Eight days later, on November 10, the population per sq. m. had increased to 28,800 Corethra larvae, 423 tubificids, 311 Chironomus larvae and 156 Protenthes larvae. Thus the Corethra had slightly more than doubled, the tubificids had nearly trebled their numbers while Chironomus had increased from 0 to 311 and Protenthes from 0 to 156.

Since sampling in Douglas Lake was confined to summer months, only a part of the seasonal cycle is adequately understood. The facts so far as they are known for that lake, agree in general trend of events, however, with those for Third Sister Lake. Quantitative data on seasonal variations of bottom fauna in Douglas Lake have been arranged according to depressions, and graphs prepared (Figs. 13-31) showing the seasonal variations in number per sq. m. within the 21 m. contour in all depressions except Stony Point depression where the curves are based on the number of organisms per sq. m. below 17 m. In each graph three curves have been drawn, one showing numbers of tubificids, a second indicating the abundance of Corethra larvae, and a third representing the total population per sq. m. In one figure (22), the curve for Chironomus has been added. The data for the three curves (tubificids, Corethra, and totals) have been selected since it was felt that these gave an adequate and lucid representation of quantitative changes going on within the deeper profundal zone.

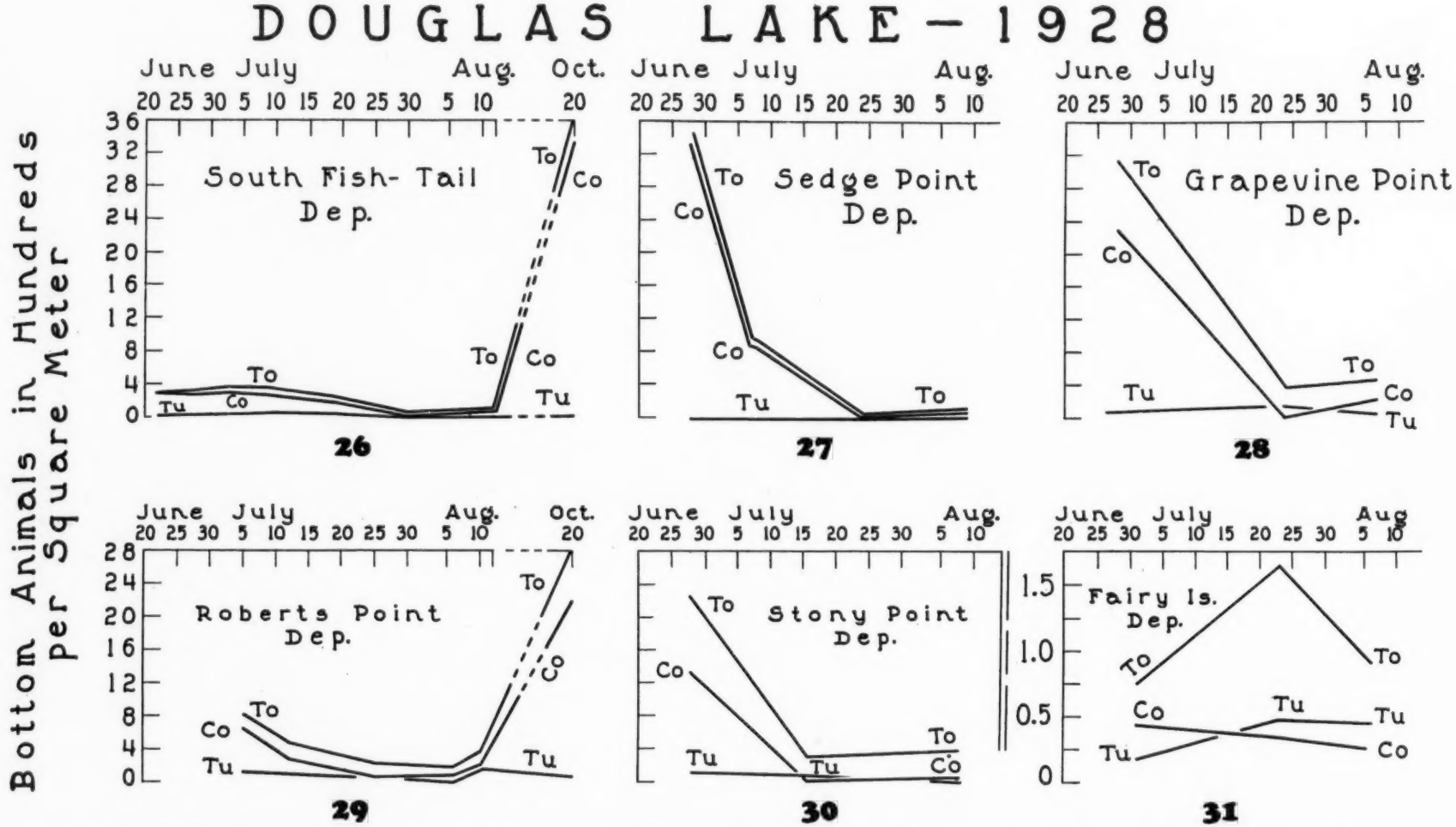


FIGS. 13-19. Quantitative seasonal variations of profundal bottom fauna within the deeper profundal zone, Douglas Lake, in 1926. The curves indicate total benthic fauna (To), Corethra larvae and pupae (Co), and Tubificidae (Tu) in number of individuals per square meter of lake bottom on the dates shown at the top of each figure.

square meter of lake bottom on the dates shown at the top of each figure.



FIGS. 20-25. Quantitative seasonal variations of profundal bottom fauna within the deeper profundal zone, Douglas Lake in 1927. The curves indicate total benthic fauna (To), Corethra larvae and pupae (Co), and Tubificidae (Tu) in number of individuals per square meter of lake bottom on the dates shown at the top of each figure.

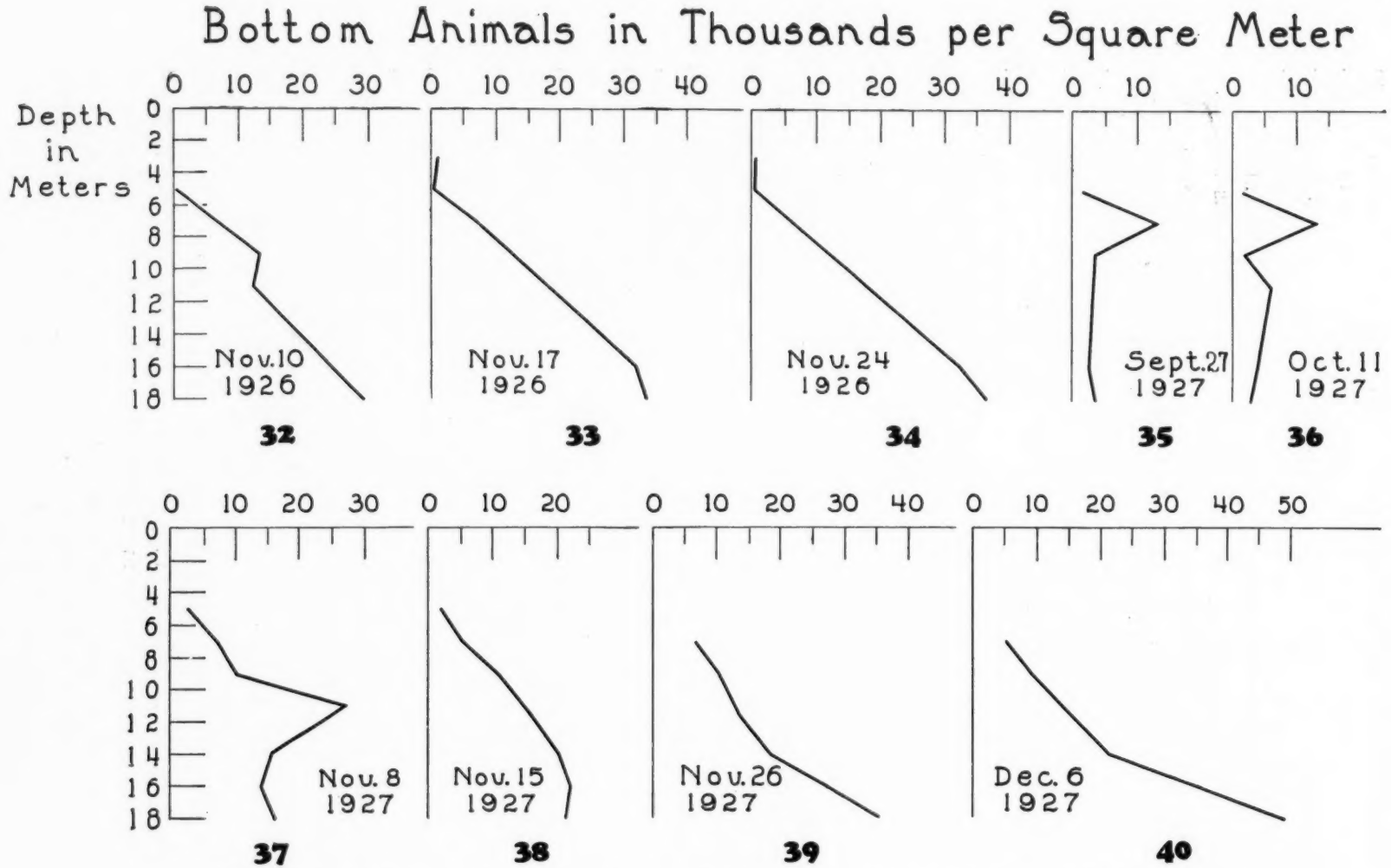


FIGS. 26-31. Quantitative seasonal variations of profundal bottom fauna within the deeper profundal zone, Douglas Lake in 1928. These curves indicate total benthic fauna (To), Corethra larvae and pupae (Co), and Tubificidae (Tu) in number of individuals per square meter of lake bottom on the dates shown at the top of each figure.

One general tendency of quantitative variations in the profundal bottom fauna was common to all depressions, namely, the gradual decline in numbers from the time the first samples were taken, usually in late June, until a summer minimum was reached some time between July 25 and August 15, depending on season and depression. There was also a nearly uniform tendency for the population to show a slow but steady increase from this time until the last samples of the season were taken. Comparison of the curves will make these facts evident. In this respect, each depression of Douglas Lake follows the same cycle as is shown by the single one in Third Sister Lake.

Season after season it became increasingly evident that each region of deep water in Douglas Lake had a certain individuality in terms of its profundal benthic fauna. The nature of this individuality is dual; partly quantitative, partly qualitative. Thus if it became necessary to have considerable numbers of any particular typical profundal form for experimental, rearing, or other purposes, the author could, with some confidence, go to a particular depression in search of the animal needed. If Corethra larvae were wanted early in the season, Sedge Point depression could usually be depended upon to yield the largest crop per unit area. A little later in the season it would be useless to visit Sedge Point depression in search of that particular animal, and tubificids were never there in large numbers. When tubificids were sought, Fairy Island depression could be expected to furnish a dependable, though never an extraordinarily numerous, supply. Grapevine Point and Roberts Point depressions were found to be perhaps the most dependable regions in terms of general productivity of typical profundal animals. In point of diversity of deep water benthic fauna, Stony Point depression soon came to be known as the most productive region. This depression also made the most sudden and pronounced recovery from the mid-summer minimum of late July and early August. Fairy Island depression, although the most dependable in production of *Tubificidae,* was, on the other hand, uniformly the least productive in point of total benthic population per unit area.

Consideration of data presented in the curves (Figs. 13-31) will make clear certain further aspects of seasonal variations within the various depressions. It will be evident, for example, that no generalization as to the exact date on which the annual minimum will occur can be made on the basis of the data now available. Perhaps it is safe to assume that the individuality of the seasons, a matter which will receive further consideration later, is greater in this respect than is the individuality of the depressions. Certainly, the records for the season of 1927 seem to point in that direction. One further tendency, of a somewhat minor nature, is clear, namely, the repeated decrease, year after year and in most depressions, of the numbers of Corethra larvae below the average per unit area for the tubificids. Finally, the very considerably lower summer totals in Douglas Lake, considering the whole



FIGS. 32-40. Depth distribution and zonation of bottom fauna in Third Sister Lake, in 1926 and 1927. The curves show the number of individuals of the total benthic fauna in thousands per square meter of lake bottom at the depths indicated (0-18 meters).

season and the whole profundal region of the lake, when compared with similar data for Third Sister Lake, are at once apparent.

The results obtained during the short study of the benthic fauna in October, 1928, are very interesting from several aspects. The relatively very considerable increase in bottom population shown graphically in the 1928 curves for South Fish-Tail and Roberts Point depressions (Figs. 26 and 29), offer rather convincing evidence that, just as in Third Sister Lake, the number of individuals per unit area within the profundal zone of Douglas Lake is suddenly and materially augmented in the fall of the year. In Douglas Lake the change appears to come approximately one month earlier than in Third Sister Lake. This may very possibly be due to a combination of two factors, the difference in latitude (represented in 300 miles) of the lakes and the perhaps still more significant difference in their size.

DISTRIBUTION

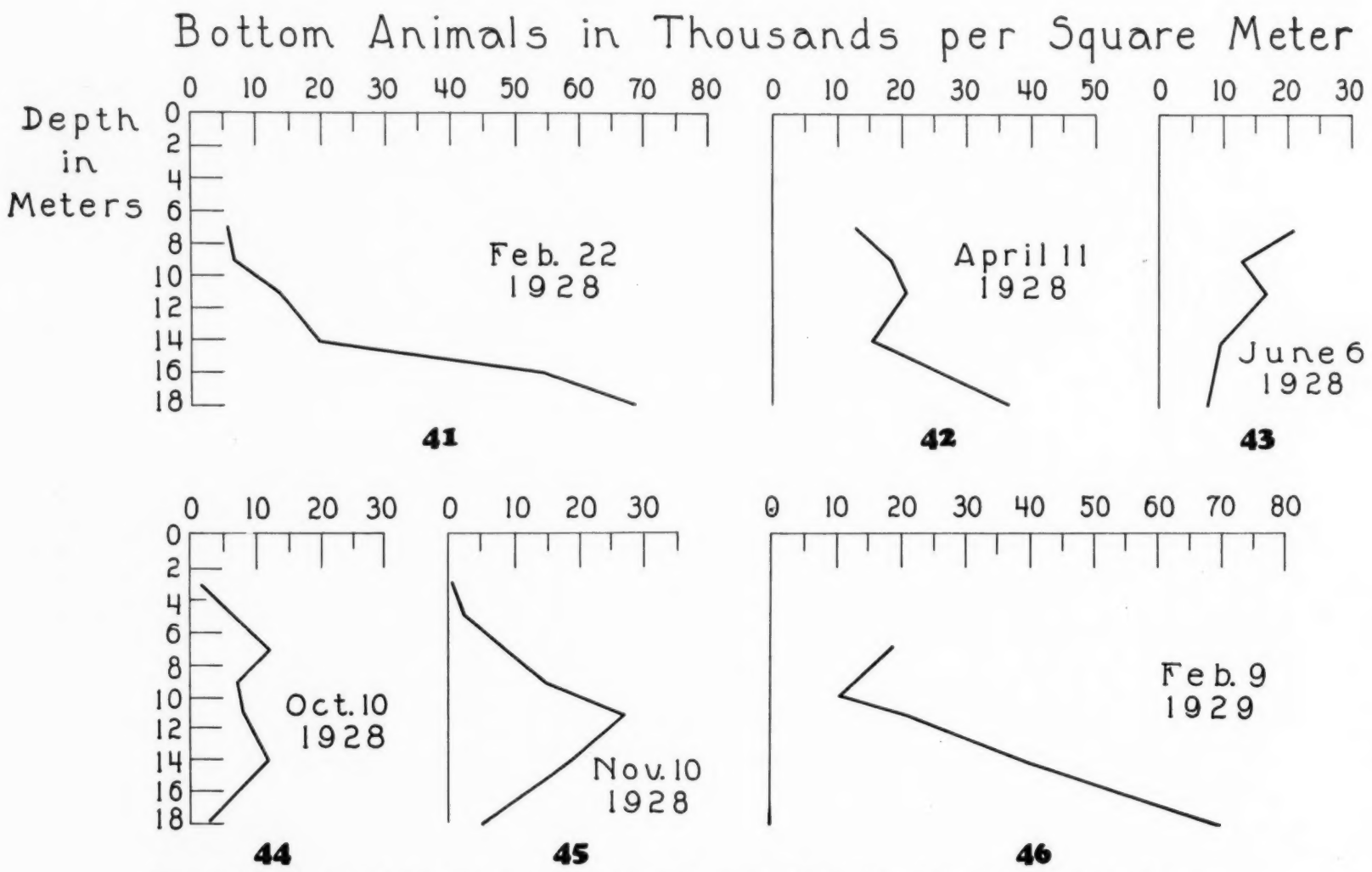
Sampling series, previously mentioned, were taken in an attempt to determine the depth distribution of the profundal fauna. Certain features at once became apparent which rendered it desirable to know whether or not there was any seasonal cycle in the character of this distribution. With this object in view, sampling series were run each summer, in various regions of Douglas Lake and at selected intervals between November, 1926 and February, 1929 in Third Sister Lake. In addition to these, a few series were taken in other lakes for purposes of comparison.

Zonation

In Third Sister Lake, the first sampling series for determining depth distribution of the benthic fauna, taken on November 10, 1926, began at the 18 m. depth and extended up the slope to the 5 m. depth. From among the subsequent series 14 have been selected and graphs prepared from the data (Figs. 32-46). The curves represent distribution, according to depth, of the total benthic fauna in terms of organisms per sq. m.

As was expected, the distribution was not uniform from deep to shallow water. During part of the year, the general trend was toward decrease of numbers with decrease of depth. At other seasons, the ratio between depth and total population per unit area of bottom was the reverse. On November 10, 1926 (Fig. 32) the number of organisms steadily decreased between 18 and 11 m.; increased between 11 and 9 m.; and again decreased sharply to 5 m. where the population was very small. At 9 m., as the curve shows plainly, there was a distinct zone in which the individuals of the benthic fauna were considerably more numerous than immediately above or below. This feature of the depth distribution, which repeatedly occurred at certain seasons of the year, will be referred to hereafter as the concentration zone.

One week after the first sampling series had been taken, another was ob-



FIGS. 41-46. Depth distribution and zonation of bottom fauna in Third Sister Lake, in 1928 and in February 1929. The curves show number of individuals of the total benthic fauna in thousands per square meter of lake bottom at the depths indicated (0-18 meters).

tained which extended from 18 to 3 m. The curve (Fig. 33) shows a nearly uniform decrease in numbers from 18 to 5 m. The previous sharp zonation had disappeared and only a slight indication of it was evident at 16 m. It will be noticed from the curve that the total population per unit area remained small from 5 to 3 m., a feature of depth distribution always found in Third Sister Lake whenever the sampling series were continued to the edge of the littoral zone. One other feature of the two curves (Figs. 32-33) is worthy of note; viz., the noticeable increase in number of organisms per sq. m. in the deeper profundal. This throws an interesting light on that sudden increase in numbers within the deepest profundal discussed in the section relating to quantitative variations. It appears that the sudden augmentation beneath the deepest water is, at least partly, brought about by obliteration of the concentration zone and a dispersal of its large population over the lake floor within the deepest regions.

The third series selected was secured on November 24, 1926. Conditions of the previous week were little changed. The curve (Fig. 34) is almost identical in general form with the preceding one except for two minor differences. It shows a still further increase in individuals within the deepest profundal zone and it is more nearly a straight line from 18 to 5 m., having lost some of the convexity, toward the right, which showed in the earlier curve. This last fact indicates a more nearly complete obliteration of the concentration zone.

Beginning with data for September 27, 1927, 12 curves (Figs. 35-46) have been prepared which depict graphically the annual cycle of events for depth distribution of the Third Sister Lake benthic fauna. On the first four dates, sampling series extended from 18 to 5 m. The series were not extended further into shallow water since previous experience had shown that few profundal bottom animals might be expected there. The next 5 series extended from 18 to 7 m.; the next 2 from 18 to 3 m.; and the last one began at 18 m. and ended at 7 m.

On September 27 (Fig. 35), the number of benthic animals at 18 m. was somewhat more than 3600 per sq. m. A slight decrease occurred between 18 and 16 m., followed by a steady but very slow increase up to 9 m. Between 9 and 7 m., there was a sudden and considerable increase from a little more than 3700 at the greater depth to almost 13,500 at the lesser. The benthic population then decreased again very rapidly to about 1400 per sq. m. at 5 m. The distribution was characteristic of the late summer and early fall conditions and the pronounced concentration zone with its maximum at 7 m. and extending from 5 to 9 m. was a thoroughly typical one.

Two weeks later, October 11, the population at 18 m. was almost exactly that of the previous date but there was a steady increase up to 11 m., followed by a sharp decrease to the 9 m. depth, and this again by another and more pronounced increase to the maximum at 7 m. As before, the popula-

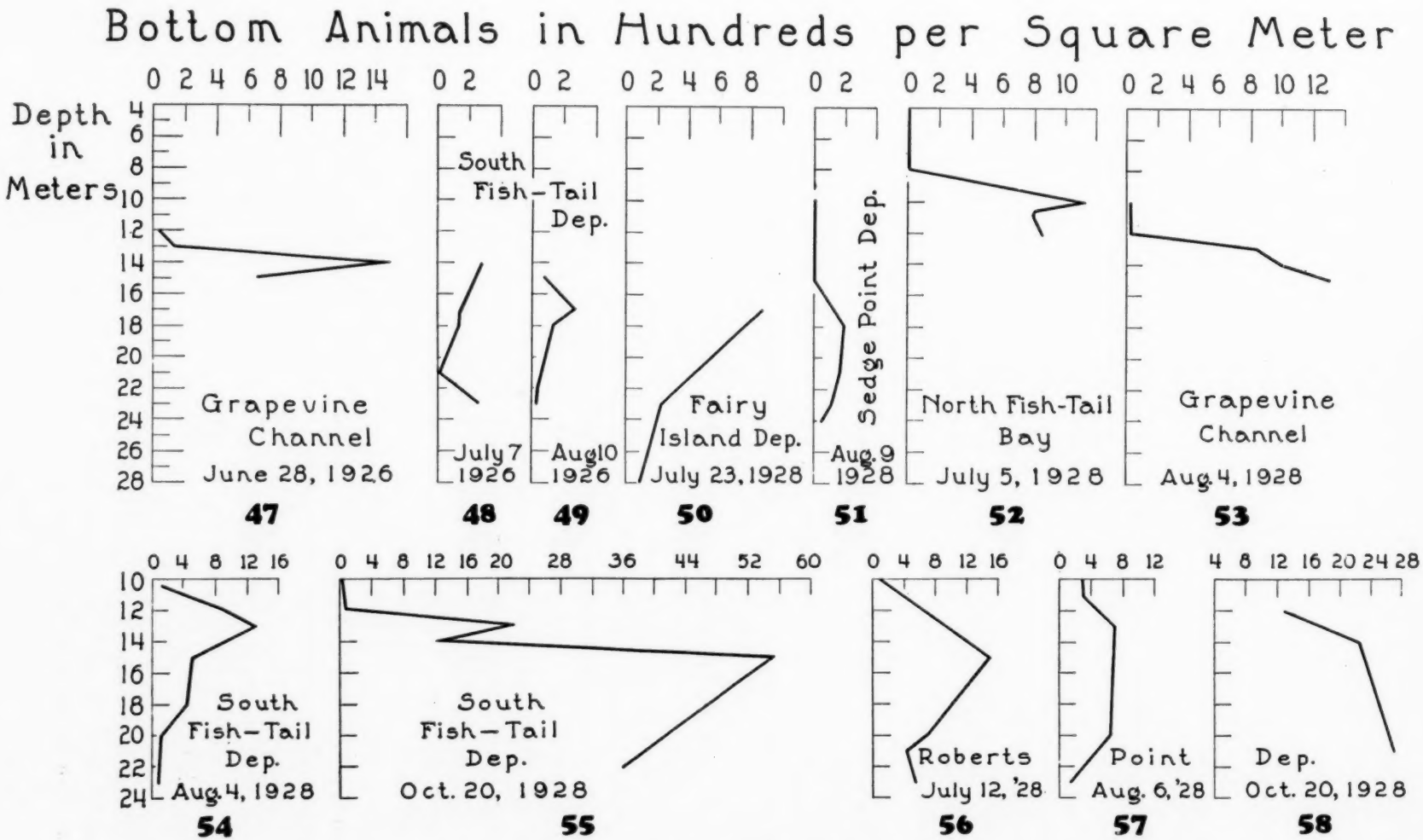
bottom at the depths indicated (0-18 meters).

tion decreased rapidly between 7 and 5 m. to a minimum at the latter depth. The curve for this date (Fig. 36), shows the same typical concentration zone at 7 m. but with an accessory one slightly developed at 11 m. Figure 37 gives data for November 8. On that date, the two concentration zones had merged into one which was much broader and of considerably greater magnitude with its maximum at 11.5 m. The mass of the benthic fauna within the profundal zone was not only increasing, but the concentration zone was shifting into deeper water and spreading out. The deeper profundal had begun to be affected by the autumnal change and its benthic population had suddenly increased to over 16,000 per sq. m.

A still more pronounced change occurred within the next week, as shown by the curve for November 15 (Fig. 38). When bottom samples were taken on that date, the concentration zone was no longer in evidence, but rather a steady decrease with decrease in depth, was found, except from 18 to 16 m. The sharp peak of the curve for the week before had disappeared and the curve now showed a gentle convexity to the right. In contrast to this condition, the next curve (Fig. 39), giving the data for November 26, shows a slight concavity on the right. The population at 18 m. on that date was still larger. There occurred a continuous, although not quite uniform, decrease as samples were taken at successively shallower depths up to the final sample at 7 m.

On December 6, a still further increase was noted at 18 m. and, as before, the decrease in population with decrease in depth was continuous (Fig. 40). On February 22, typical midwinter conditions prevailed and the annual maximum occurred at 18 m. A sharp decline in population was found up to 14 m. and thence to 7 m. the decrease was less abrupt, although uninterrupted (Fig. 41). If the curves for November 26, December 6 and February 22 be compared, it will be seen that the concavity toward the right steadily increased and that the population below 14 m. was continuously growing. In effect, this had, by the last date, produced a concentration zone extending from 14 to 18 m. with its maximum at the lower depth.

Early spring conditions are revealed by Fig. 42, which is the curve for April 11. The maximum was still at 18 m. where the benthic population was more than 36,500 per unit area. However, a return to summer conditions with a concentration zone in shallower water was evidently on the way. The curve shows plainly the beginnings of such a movement, with the incipient concentration between 14 and 7 m. and *its* maximum at 11.5 m. The curve for June 6 (Fig. 43), offers further evidence that the April data were collected just at the time when the summer concentration zone was beginning to form. Unfortunately, the June 6 series was not extended farther up the slope than the 7 m. depth. A clearly defined concentration zone was present between 14 and 9 m. and the maximum was, as usual, at 11.5 m. The peak found at 7 m. shows the presence of another concentration zone above the



FIGS. 47-58. Depth distribution and zonation of bottom fauna in Douglas Lake. The curves show number of individuals of the total benthic fauna in hundreds per square meter of lake bottom at the depths indicated (0-28 meters).

9 m. level, probably with its high point where the series was discontinued. If Fig. 36 is compared with Fig. 43, it will be seen that from 18 to 7 m. the curves are much alike, except that the magnitude of the population represented by the former is much less than that in the June curve. If the similarity of these curves is considered in conjunction with the data from bottom samples taken at various depths, but not in series, during the springs of 1927 and 1928, then it seems almost certain that had the series of June 6, 1928 been continued to 3 m., the curve would have declined sharply from the 7 m. maximum to a very low total at 5 m. and continued low to 3 m.

In the fall and early winter, 1928, the cycle of events which had taken place in the depth distribution of the bottom animals during the two preceding autumns was repeated. Curves prepared from the data for 3 dates (Figs. 44-46) show: (1) the inception of the autumnal shifting in the concentration zone; (2) the merging of the intermediate, double concentration zone into a broad and sinking single one; and (3) the fully established midwinter concentration in deep water.

The curves for October 10 and November 10, 1928 are comparable with those of October 11 and November 8, 1927. Perhaps the two for the fall of 1928 may represent intermediate stages between the two for 1927. Other such interpolations of curves might be made, and postulates drawn as to detailed changes in the annual cycle of the concentration zone. It must be remembered, however, that this would be largely a matter of conjecture, and furthermore that the transition from one phase to another is not exactly duplicated as to detail in each succeeding year.

The restriction of the sampling almost entirely to the summer months in Douglas Lake, together with the limitations placed on intensive study of any one depression by the necessity of a single investigator spreading his efforts over what amounts, in effect, to 6 lakes within one, have resulted in a much less complete series of curves than was obtained for Third Sister Lake.

Figures 52 and 54 illustrate depth distribution of total bottom population per unit area in North and in South Fish-Tail Bays respectively. A feature of the depth distribution, which occurs repeatedly in Douglas Lake particularly during the first half of the summer, is shown in the curves of Figs. 48, 52 and 56. This feature is the development of a pronounced concentration zone, usually within the lower sublittoral, followed by a sharp decline in population beginning just above the upper limit of the hypolimnion, and another slight increase as the deepest region is approached. That a concentration zone is present in various regions of Douglas Lake, although at a greater depth than in Third Sister, is demonstrated by the figures in general and particularly well by Figs. 47, 49, 54, 55 and 56. The curve for Roberts Point depression on July 12, 1928 (Fig. 56), is typical of the depth distribution which is probably most frequently found in the major depressions of Douglas Lake during June and July. That the truncated form of the curve

for August 6 of the same year, and in the same depression is not due entirely to absence of data for depths between 13 and 20 m. on that date, seems to be indicated by bottom fauna counts between those depths on comparable dates in that and other years. Bottom samples taken at random between these 2 depths seldom yielded as high a count during August as during July.

Sedge Point depression usually showed a different sort of depth distribution than was typical in the other major regions of deep water. The curve for August 9, 1928, illustrates this difference, although it should be pointed out that, occasionally, earlier in the summer, slight indications of a concentration zone were found even in Sedge Point depression. No complete sampling series, extending from deep to shallow water, was taken in Fairy Island depression during any one day. Figure 50, however shows the results of a partial series which extended between 28 and 17 m. Since the population almost certainly decreased rapidly between 15 and 10 m., it may be regarded as probable that a well developed concentration zone occurred between the depths of 20 and 10 m. This is further substantiated by results of sampling done at other times in the sublittoral zone on the edge of, or adjacent to, the Fairy Island depression. In Grapevine Channel, there was usually a well developed concentration zone with a region of lower productivity below. However, as on August 4, 1928 (Fig. 53), this was not always the case, and, on several occasions, the population decreased sharply and nearly uniformly from the deepest water to a depth of about 12 m., above which few bottom animals were ordinarily found.

Some indication of the autumnal depth distribution of the Douglas Lake bottom fauna is given by the two curves for October 20, 1928 (Figs. 55 and 58). The curve for Roberts Point depression is based on data from three depths only and consequently may not reveal the whole story of distribution on that date. It may be pointed out, however, that if the distribution was of the sort to be expected (judged by what is known for both Douglas and Third Sister Lakes), then data for those depths at which samples were actually taken would yield a curve like the one obtained, even if such data were selected from a very complete series instead of from an incomplete one. The curve for the sampling series of the same date, but taken in South Fish-Tail depression and extended on into Grapevine Channel, is much more dependable, since the data are more complete. There, the distribution was much the same as that found in Third Sister Lake during the autumnal obliteration of the concentration zone and the dispersal of its numbers over the bottom in the deeper water.

Seasonal Distribution

The graphs prepared to show zonation indicate only total number of organisms per sq. m. The seasonal and qualitative aspects of depth distribution, which may be considered together, require more detailed presentation of

the data. Therefore, a series of tables has been prepared, in which the qualitative and the seasonal aspects have been accentuated. Tables XX-XXVII present the data primarily from the seasonal aspect for different depths in Third Sister Lake. In Table XXVIII the data are arranged to show, more clearly, the qualitative phase of seasonal and depth distribution of bottom animals.

Not only does the total number of benthic organisms per unit area vary with depth and with the season but each species included in the total has distinctly individual variations of the same two sorts. Thus, Corethra larvae were found to vary in the following ways: (1) the total number in the whole lake varied with the season, (2) the number per unit area of bottom varied, (a) with the season differently at each different depth (Tables XX-XXVII) and (b) with the depth differently at different seasons of the year (Table XXVIII). Computations of the standing crop at various seasons, which have not been included herein but may be made with the data given, show statement (1) in the preceding sentence to be a fact. The columns for Corethra in Tables XXI, XXV, and XXVI will illustrate what is meant by (2, a) above. In the same way, (2b) above will be evident if the data for June 6, 1928, November 10, 1928, and February 9, 1929 (Table XXVIII), are compared. A further factor affecting the numerical variations of Corethra larvae at different seasons and depths is the occurrence of considerable numbers of these larvae in the limnetic habitat.

What has been pointed out for Corethra could be repeated for each of the other types of bottom animals. Chironomus larvae vary greatly with the depth at practically all seasons, although the variation was more abrupt at certain times. When considered alone, they also show a pronounced concentration zone, as for example, on October 11, 1927 (Table XXVIII). The virtual restriction of their concentration zone to the border line region between profundal and sublittoral seems very characteristic of these larvae in Third Sister Lake. Table XXV illustrates clearly the seasonal numerical fluctuations even within the region of their own greatest concentrations. A variation from 45 to 6969 per sq. m. is certainly a significant change. The maxima for Corethra and Chironomus larvae far surpassed those for Protenthes, yet there were instances, particularly in the shallower depths, when Protenthes larvae outnumbered both the other dipterans combined.

The *Tubificidae,* considering both time and place, were perhaps the most uniform in distribution of any group within the typical profundal bottom fauna. The *Tubificidae* column in Table XXVIII shows that they were always present at all depths listed and on all occasions, except between 3 and 4 m. on November 17 and from 3 to 6 m. on November 24, 1926. In the same table, data for November 8, 1927, depicts the clearly developed concentration zone of these annelids on that date at 11.5 to 13 m. The maximum number per unit area found during this investigation was in Third Sister

Lake, at 11.5-14 m. on April 11, 1928, when the tubificid population amounted to 18,132 per sq. m. (Table XXIII). Populations of *Tubificidae* in excess of 9,000 per unit area were found at various times, between 15 and 7 m. (Tables XXII to XXV). Above the latter depth, they were never known to exceed 400 per sq. m. in this lake (Tables XXVI, XXVII).

In Table XXVIII, data on the abundance and on depth and seasonal distribution of the Hydracarina in Third Sister Lake will be found. Combinations of these data with those given in the annotated list (p. 256) will yield information on relative abundance and occurrence of various genera and species. Sharply defined zonation frequently characterized the depth distribution of these mites. The same statement applies equally to the organisms listed in the "All Others" column. Many minor features in the zonal, seasonal, and qualitative distribution of the bottom fauna were observed and several of these are illustrated in the tables. In addition, tabulation of the data has revealed other features not discussed.

The seasonal and qualitative phases of depth distribution, as observed in Douglas Lake, are shown in Table XXIX. Columns showing the occurrence and distribution of *Sphaeriidae* and Nematoda are included in that table, in addition to those given in the other tables. As expected, considering the time of year when the sampling was done, the bottom fauna counts in Douglas Lake were greatly below the maxima found in Third Sister Lake. Similarly, the magnitude of the variations was less than in Third Sister Lake. However, the same generalizations with regard to distribution apply. And to the list of various ways in which each organism differed at different seasons and depths in Third Sister Lake, still another may be added for Douglas Lake; *viz.*, each kind of animal in the benthic group varied with the season and with the depth, differently in different depressions. This fact was well illustrated by the sampling in South Fish-Tail Bay on August 4, Roberts Point depression on August 6, and Sedge Point depression on August 9, in 1928 (Table XXIX). In South Fish-Tail Bay, Corethra larvae were taken on the date just mentioned from 12 to 23 m., with a concentration zone at 15-18 m.; in Roberts Point depression between 10 and 22 m., with a broader concentration zone, 11-20 m.; and with the maximum at 13 m.; while in Sedge Point depression no larvae were collected above 18 m. and a true concentration zone was lacking.

Depression individuality was even more distinctly shown by the comparative distribution of Protenthes larvae on the same dates. In South Fish-Tail Bay, these larvae extended from 10.5 to 18 m. with a sharp concentration zone at the 13 m. level; in Roberts Point depression they showed an irregular distribution between 10 and 22 m.; while in Sedge Point depression they were taken only at the 20 m. level and there in very small numbers. The discussion might be extended to the other forms and dates but the two examples described will serve to illustrate the point.

Both the *Sphaeriidae* and the Nematoda showed considerable variation in their distribution at different depths in the different depressions. The most significant data, from the seasonal aspect, obtained in Douglas Lake was that for October 20, 1928, in Roberts Point and South Fish-Tail depressions (end of Table XXIX). Not only were the quantitative changes mentioned before apparent, but marked changes of a qualitative nature and in depth distribution had occurred since these two depressions were last visited, late in August. If the data for these dates are compared with those for the same depressions on other dates, as for instance those for South Fish-Tail depression on June 22, 1928, or July 7, 1926, and those for Roberts Point depression on July 12 or August 6, 1928, the seasonal and qualitative variations are evident.

Maple Point depression, Douglas Lake (Fig. 3) was practically ignored throughout this investigation since occasional sampling there never yielded any bottom organisms. This was in sharp contrast to results obtained in other depressions at comparable depths and dates, as for instance, on August 3, 1926, at 17 m. in Roberts Point depressions where Corethra, Chironomus, Protenthes, *Tubificidae*, *Sphaeriidae*, Nematoda and Sialis were all represented. The bottom near the center of North Fish-Tail Bay (Station 60) and at a position about midway between the two points at the entrance to the bay (Station 70) usually yielded a fairly large summer benthic fauna. Station 60 frequently was the more productive of the two although this was not always the case. Hydrachnids were more often taken at these stations than elsewhere in the lake. Grapevine Channel was usually a productive region in terms of bottom fauna. More nematodes were taken in the Channel than at any other point. On several occasions, samples were taken in the various interdepression regions, as for instance, between Station 30 and Station 50 and the counts of animals often were higher than in samples from the same depth within the edges of the major depressions (Table XXX).

Kirkville Green Lake was unique in many ways, particularly in regard to bottom fauna. Between the depths of 1 and 61 m. not a single specimen of Corethra, Chironomus, Protenthes or *Tubificidae* was taken in 145 bottom samples (Table XXXI). Hydracarina, Gastropoda and Crustacea were found down to depths of 5 m. Below that depth no bottom animals whatever were collected. So far as macroscopic benthic animals are concerned, by far the larger part of the lake floor was absolutely barren.

ECOLOGICAL RELATIONS

FACTORS INFLUENCING VARIATION AND DISTRIBUTION

The environmental factors which affect seasonal variations, both qualitative and quantitative, and depth distribution of profundal bottom animals are of three general kinds: biological, chemical and physical. Specifically, light, temperature, water movement, chemical content of superimposed water, char-

acter of bottom, food, enemies and the life cycles of the animals involved are among the factors which might affect some or all of these activities of the benthic animals. The two interrelated phenomena of seasonal variation and distribution are both affected by the same set of ecological factors. Hence it is logical to consider them together.

Bottom Characteristics

Within the regions sampled, the various types of bottoms might be classified as sand, marl, clay and mud. The mud bottom was most typical of the profundal and lower sublittoral. However, the nature of this type of bottom deposit was not always uniform. As previously shown, the mud varied in amount of water, organic detritus and coarse debris, which it contained. It also varied in color, odor, rate of settling when stirred up, and in its tendency to rise to the surface and form floating masses.

Bottom deposits provide support and protection for the benthic fauna. With the exception of Corethra larvae and the adult stages of this and other insects the profundal benthic animals spend their entire existence on or in the bottom. Tube-building and burrowing of chironomid larvae and tubificids are well known.

The question was raised whether the Ekman-Birge dredge missed some of the bottom animals in the muddy water above and in the deeper mud beneath the level sampled. This possibility was thoroughly investigated early in the work. Much direct observation in the field and certain laboratory experiments demonstrated conclusively that, with rare exceptions, the bottom animals occupy the middle strata of the samples taken with the Ekman-Birge dredge. Neither the watery layer at the top nor the deeper mud layers at the bottom of these samples contained benthic animals.

In a few instances, Corethra larvae were kept for a time in bottles containing a concentrated live plankton collection. Under such conditions, these larvae soon showed a digestive tract filled with plankton organisms. In aquaria, with very little plankton but plenty of profundal mud, Corethra larvae frequently fed on the detritus. On several occasions, dissection of Chironomus, Protenthes and tubificids showed that all had been feeding on organic detritus. Ordinarily, there appeared to be more fine sand grains in the digestive tract of the tubificids than in those of the insect larvae. This fact seemed to suggest the possibility of a certain amount of selection by the larvae but it may, of course, have been accidental. Similar observations have been made by other investigators on the bottom animals in other waters.

That the character of the bottom deposits affects the nature and distribution of the bottom fauna was frequently demonstrated by sampling series. On sandy bottoms, as in Maple Point depression and on the sandy slopes of other depressions of Douglas Lake (Tables XXIX-XXX), bottom animals were few or wholly absent. When the bottom was composed of marl or a

mixture of sand and marl, as for example, in the shallower depths of Kirkville Green Lake (Table XXXI), a benthic fauna predominated by the Mollusca occurred. However, mud forms by far the greater part of the bottom deposits in the lakes studied and on the mud bottoms were encountered both the largest and some of the smallest populations. The number of animals taken in such substrata varied from the enormous populations of Third Sister Lake to complete absence in the deeper waters of Kirkville Green Lake. In Third Sister Lake, the profundal bottom deposits are a mixture of clay and black, organic detritus. In Douglas Lake, the bottom within the profundal zone is more largely black detritus and very finely divided sand, and there the numbers of bottom animals were intermediate. The character of the deeper mud deposits in Kirkville Green Lake is certainly different from that of the mud in either Third Sister or Douglas Lake. In fact, it appears to resemble the dystrophic lake deposits of Europe—the *"Torfschlamm"* or *"Dy"* of European writers. The bottom muds of Third Sister and Douglas Lakes, on the other hand, appear to be more like the *"Faulschlamm"* or *"Gyttja"* of the same investigators.

In Kirkville Green Lake, the only bottom fauna taken was from the shallow water where the bottom deposits were mixed with sand, marl, or clay. In Third Sister Lake, the bottom fauna was greatly decreased within the 5-3 m. zone (Figs. 32-46; Tables XXVII-XXVIII) and in this region, the bottom had a very large proportion of coarse, decaying plant remains and practically none of the clay-detritus of the profundal and lower sublittoral. Bottom deposits within the profundal region of Douglas Lake varied somewhat in different depressions, but these variations were always of a secondary nature. In all depressions, the same black detritus was present in considerable quantities. Likewise, variations in the profundal fauna, both qualitative and quantitative, occurred in different depressions, but these seemed not to be clearly correlated with differences of bottom mud. Certainly no such correlations between variations within the fauna and bottom deposits occurred within the true profundal region of Douglas Lake as within the sublittoral and the lower littoral. There the pronounced changes in character of bottom deposits were clearly reflected in changes within the fauna.

Physical-Chemical Factors

Physical-chemical conditions of the benthic habitat are controlled largely by the superimposed water. The great significance of thermal-chemical stratification and stagnation is accordingly evident. Consequently it was thought necessary to make frequent, complete vertical series of physical-chemical determinations from surface to lake bottom. Such determinations were ordinarily made where the water was deepest.

Three special series of determinations were made in Third Sister Lake to determine whether or not the values found for any given factor at a particular

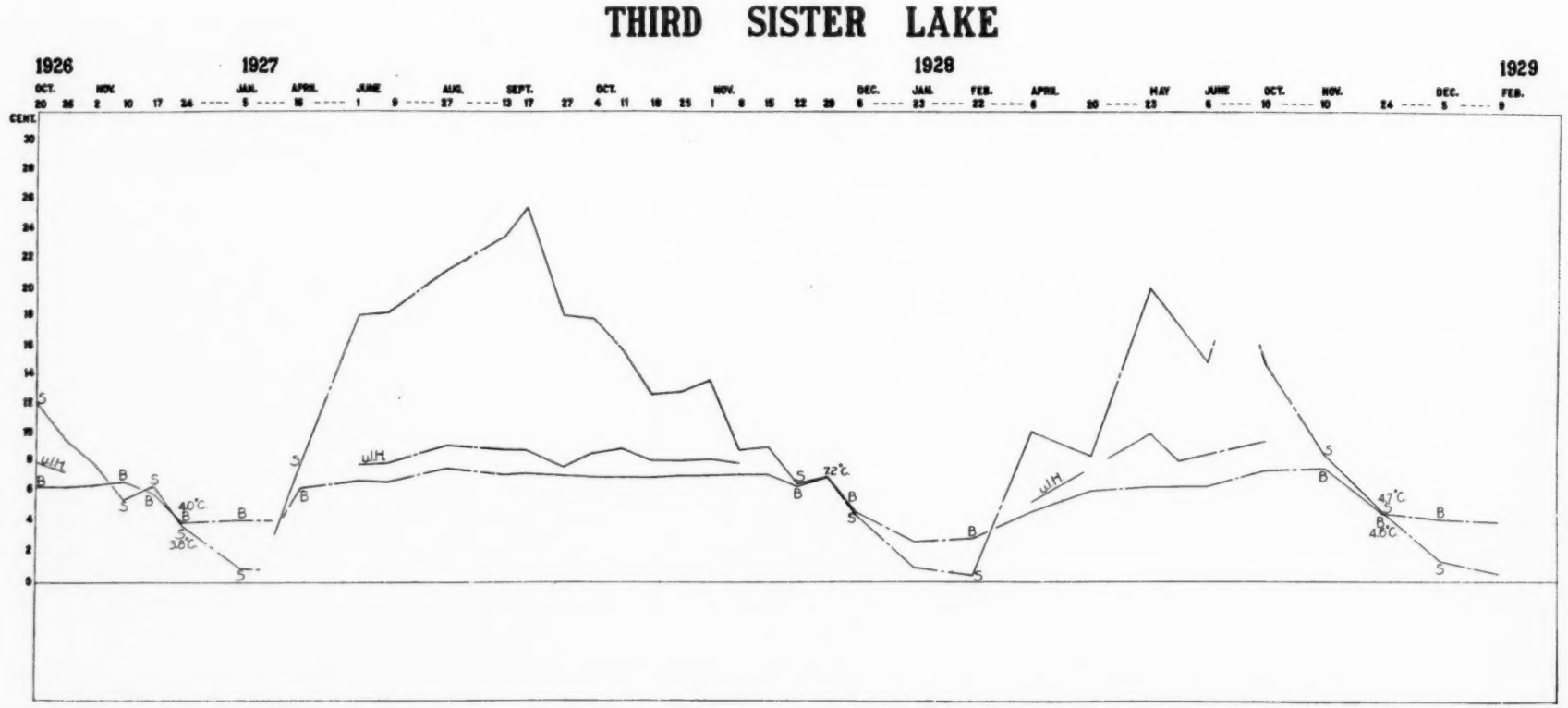


FIG. 59. Seasonal variation of water temperature in Third Sister Lake at the surface (S), upper limit of the hypolimnion (u.l.H.), and bottom (B) in degrees Centigrade.

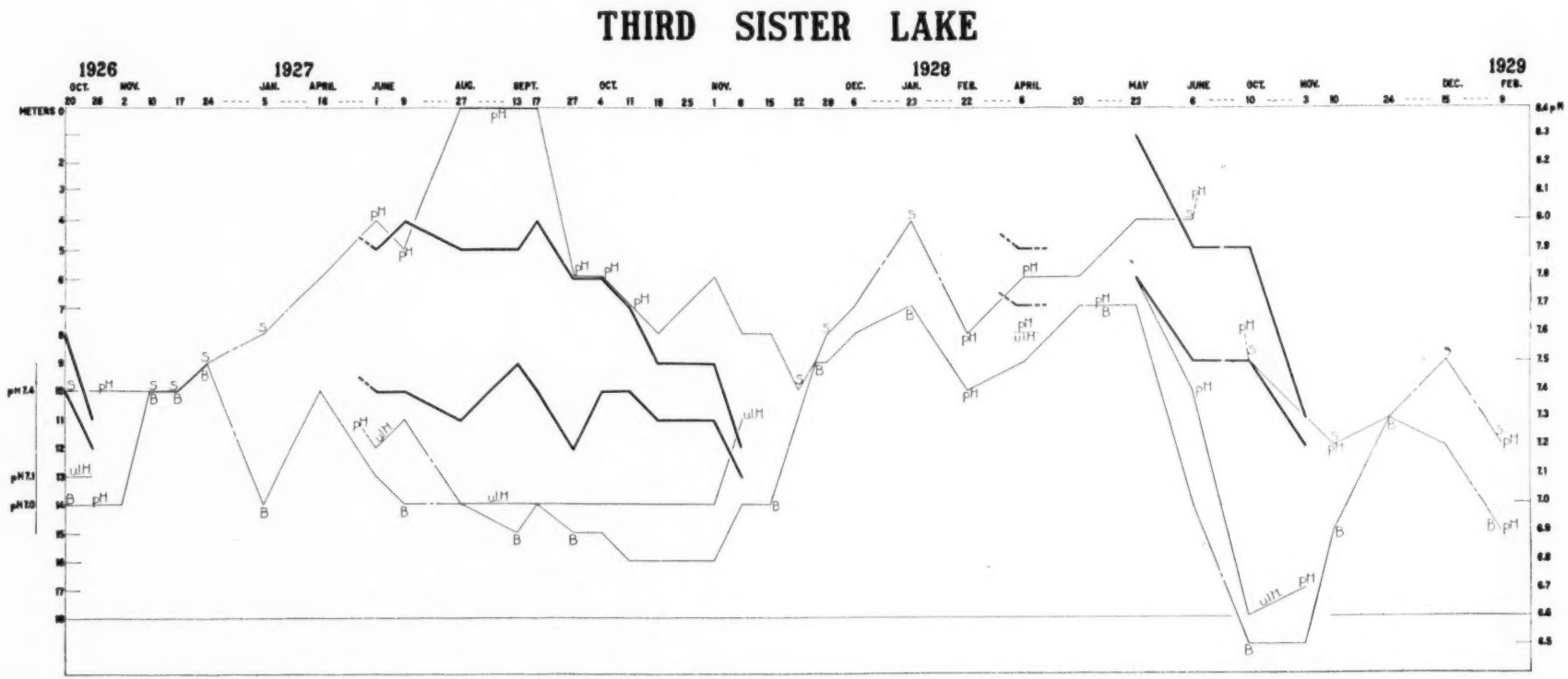


FIG. 60. Seasonal variation of pH in Third Sister Lake at the surface (S), upper limit of the hypolimnion (u.l.H.), and bottom (B). The fluctuations of the pH are shown by the light lines. Position, extent and duration of the thermocline is indicated by the heavy lines; the upper heavy line thus indicating the level of the upper limit of the thermocline and the lower giving the bottom of the thermocline at any date.

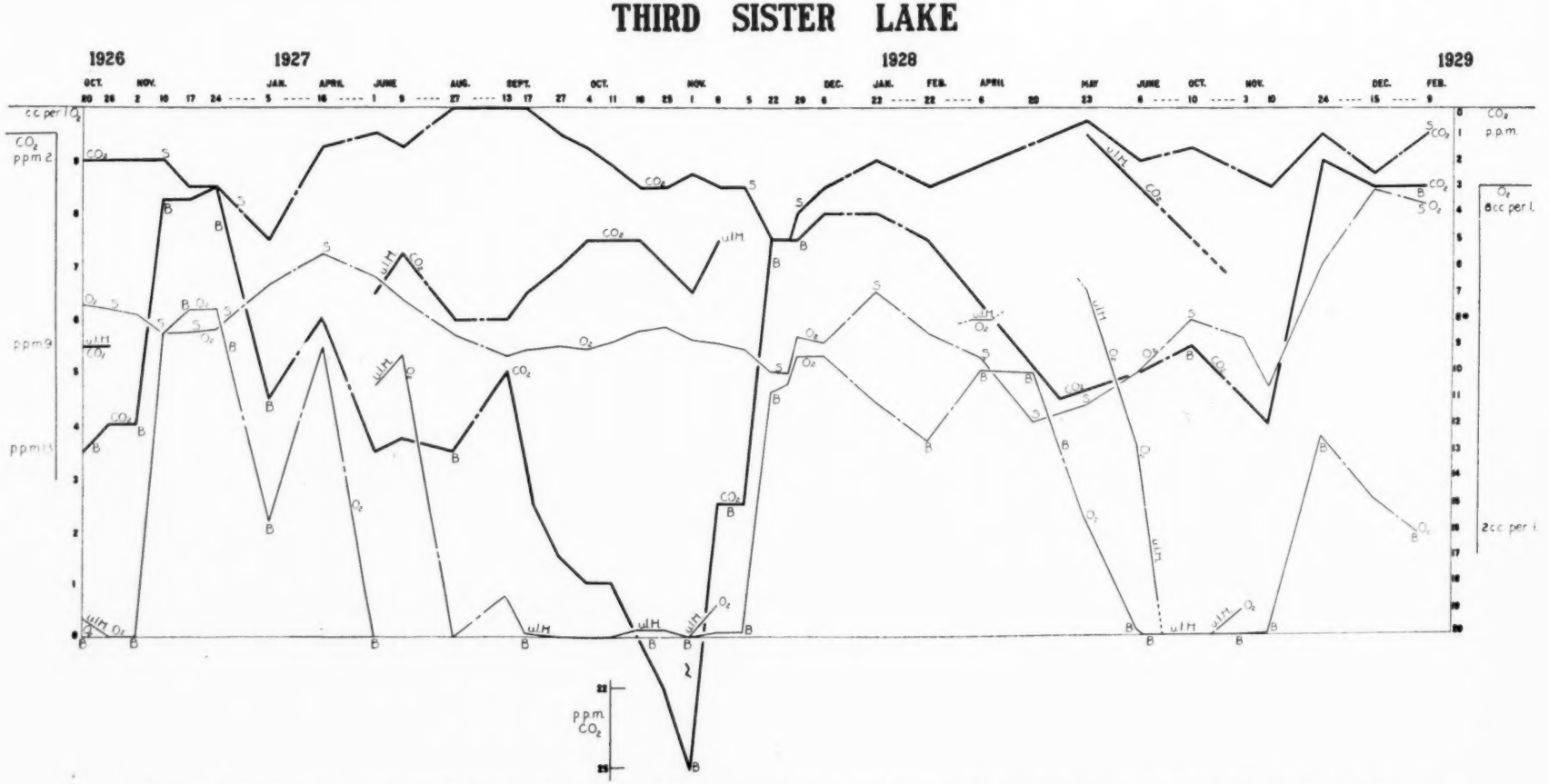


FIG. 61. Seasonal variation of dissolved oxygen (0_2) in cc. per liter and free carbon dioxide in parts per million at the surface (S), upper limit of the hypolimnion (u.l.H.) and bottom (B) in Third Sister Lake.

depth over the deep water were the same where that stratum came in contact with the sloping sides of the basin (Table XXXII). In these analyses, the samples were taken just above the bottom at selected depths. The first water drawn from the sampler was discarded on account of its turbidity and the clear water used for analysis. On the same days that these analyses of bottom water were made, complete vertical physical-chemical series were taken where the water was deepest, (Table XXXIII). Comparison of Tables XXXII and XXXIII will show that data for any given stratum in the deeper water always varied slightly from those taken where the same stratum was in contact with the slope of the lake basin. However, the differences found were never great. Oxygen content of the water was usually a little lower at the periphery of a particular stratum than in the deepest water, free carbon dioxide was higher, and pH readings usually showed the water to be slightly less alkaline. Temperature differences were always very small. These very slight dissimilarities might easily have been due to the influence of the bottom mud. The results indicated, however, that physical-chemical conditions of the water for each stratum of the lake (or of the individual depression in Douglas Lake) were very nearly the same where that stratum came in contact with the lake floor as over the deeper water. Thus, in considering the environmental conditions on the lake floor, at any particular depth, *e.g.*, at 7-8 m. in Third Sister Lake, it is safe to use the physical-chemical data for the same depth from the vertical series taken on the same date although such data were secured over the deepest water. Table XXXIV gives data for physical-chemical determinations in various extra-depression regions and shows that in Douglas Lake stratification was rarely found outside the depressions. These determinations were all from shallow water and are thus comparable with those from near the surface (0-10 m.) above the depressions.

A pronounced correlation between seasonal qualitative-quantitative variations of profundal bottom fauna within the true profundal zone, Third Sister Lake, and the seasonal cycle of physical-chemical factors was evident throughout the investigation. Figures 59-61 present a summary of the seasonal cycle of physical-chemical factors between October, 1926, and February, 1929. The curves in these figures show surface, upper limit of hypolimnion and bottom values for pH, dissolved oxygen in cc. per 1., free carbon dioxide in p.p.m., and temperature in degrees centigrade. Figure 60 also indicates the position, extent and duration of the thermocline. These curves show two summer stagnation periods (1927 and 1928) and the close of a third (October, 1926), three periods of typical winter conditions (January, 1927; January, 1928; February, 1929), and five autumnal and vernal overturns.

If seasonal variations of bottom fauna within the profundal zone (Fig. 12) are compared with data presented in these curves, it will be seen that the three annual minima for bottom animals (late summer and early fall, 1926, 1927, 1928) coincide with periods of extreme physical-chemical stagnation.

During late October, 1926, the dissolved oxygen had entirely disappeared from the whole hypolimnion; the pH varied from 7.1 to 7.0; and the free carbon dioxide from 9 to 13 p.p.m. within the same region of the lake. Coincident with this condition, there occurred a minimum of benthic animals in this zone. That similar coincidence of severe stagnation and reduced profundal bottom population beneath the deeper waters existed during the late summer and early autumn of the other two years, is shown by the curves. The profundal fauna within the 18 m. contour (Fig. 12) also manifested three clearly developed periods of maximal abundance. These maxima occurred when temperature in the profundal zone was near 4.0 degrees Centigrade, dissolved oxygen not less than 2.0 cc. per 1., free carbon dioxide varying between 3 and 11 p.p.m., and pH between 7.7. and 6.9.

The autumnal overturn greatly affects the qualitative and quantitative aspects of the benthic fauna as is strikingly shown by the records for Third Sister and Douglas Lakes. The sudden increase in bottom population beneath the 18 m. contour in Third Sister Lake each autumn during November has already been pointed out. Reference to Table XX will show that between October 26 and November 2, 1926, the total population per sq. m. within the 18 m. area increased from 7,725 to 12,836. Figures 59-61 show that a thermocline was present on the former date but that on the latter the autumnal circulation period had begun. Within the next eight days, the bottom population rose to 29,688 per sq. m. and within that time the whole lake had become thermally and chemically uniform. Again, on November 1, 1927, the count for bottom animals within the 18 m. area was 7,360 per sq. m. while one week later, it was more than 16,200, and by November 21 the population exceeded 37,700. During this same three weeks, the thermocline had been obliterated, the whole lake set in circulation, and the summer stagnation relieved. Once more in November, 1928, the bottom population at 18 m. rose from 4,916 to 30,648 per sq. m. within 14 days and coincidently the fall overturn had set in.

In Douglas Lake, the only fall records are those for October 20, 1928 (Tables VI, XI, XXIX). On that date, both South Fish-Tail and Roberts Point depressions were practically uniform, from surface to bottom, thermally and chemically. Both depressions were thermally stratified when the last summer readings were taken, and showed well developed, typical summer stagnation, with a hypolimnion wholly devoid of dissolved oxygen. Also, the bottom fauna counts were low in both depressions. On October 20, however, the bottom populations found in these depressions were by far the highest which have been recorded for this lake. In Roberts Point depression, the circulation had become more complete and correlated therewith was a wider dispersion of bottom fauna than in the South Fish-Tail depression.

A further correlation between degree of stagnation within the hypolimnion and variations within the profundal bottom fauna is to be found in the qualitative and quantitative differences between the bottom fauna in different

depressions during the same summer. Records of physical-chemical determinations show that South Fish-Tail, Sedge Point, Roberts Point, and Fairy Island depressions are, with rare exceptions, thermally and chemically stratified throughout each summer and that stagnation becomes rather pronounced in each of them by late July or early August. The faunal records (Tables XIV-XVII) also show that these same depressions rarely have any species other than the typical profundal fauna within their deeper profundal regions during the summer, and that these types also dwindle to an annual minimum in late summer. On the other hand, Grapevine Point and Stony Point depressions frequently show a complete lack of thermal-chemical stratification (Tables VII, X) and correlated with this the faunal records (Tables XVIII, XIX) show, not only a greater variety of species present in the deeper profundal region ("All Others" column in tables), but also more individuals among the typical profundal species than are present in those depressions characterized by a stagnant hypolimnion during the summer months.

Formation and obliteration of the bottom fauna concentration zone in Third Sister Lake was found to follow the seasonal cycle of stagnation and circulation. As summer stratification set in the concentration zone began to form. However, this concentration of benthic animals was not simply a response to onset of summer stagnation. This is evident from the fact that it had begun to form as early as April 11, 1928 (Fig. 42), and by June 6 of that year (Fig. 43) had reached its typical summer form. Other factors contributing to the complex which brings about this zonation of bottom fauna are considered on following pages. Reference to Figures 59-61 will show that on April 11 there was an abundance of dissolved oxygen throughout the hypolimnion and that while a thermocline was present, it later disappeared. It can hardly be said, then, that the stimulus which had initiated formation of the concentration zone was the beginning of summer stagnation. Mere formation of a thermocline could not be supposed to greatly influence, directly and so quickly, the distribution of benthic animals. But it seems just as evident, when the data are all considered, that, once the concentration zone is established, development by that time of profundal stagnation does act as a barrier to prevent dispersal of this fauna over the entire profundal region of the lake floor. Consequently, when the concentration zone had reached its typical summer form by June 6, 1928, the population of that zone was deterred from spreading over the whole lake floor by the comparatively severe stagnation within the deeper regions of the lake (Figs. 59-61).

With the coming of the autumnal circulation (Figs. 59-61) the concentration zone began to shift into deeper water (Figs. 44-46), or in other words, as the thermocline gradually sank more and more of the profundal bottom was released from stagnation, and as this release progressed the bottom animals followed it down the slope. Soon the lake became nearly homothermous from top to bottom and the whole body of water was set in circulation.

That this circulation is not a mere figure of speech, but rather, that actual currents of considerable strength are set up (under the combined influence of the strong fall winds and the now nearly uniform density of the water) which sweep along the profundal lake bottom, was clearly demonstrated during November, 1926, 1927 and 1928. Furthermore, that these currents are of considerable direct, as well as indirect, importance in the life of the bottom animals, was indicated by certain incidental but significant observations which the writer was able to make each autumn.

During much of the time between October, 1926, and December, 1928, experiments were in progress which required keeping glass containers on the lake floor within the deepest region of Third Sister Lake. Such containers were placed in cubical baskets constructed of coarse mesh wire netting. These baskets rested on the upper surface of the firm mud bottom. Each autumn and spring when the overturns were in progress the baskets, when brought to the surface, contained quantities of tree leaves, bits of shoreward vegetation, twigs, and on many occasions Odonata and Ephemerida nymphs. On November 24, 1928, one basket contained a small sunfish, alive and active, and, in addition, several Odonata nymphs. On November 29, 1927, several active leeches *(Erpodella punctata* and *Glossiphonia complanata),* many damsel-fly nymphs, and a few dragon fly nymphs were found in one basket. Another basket contained a small (6 cm.) *Ameiurus natalis* in addition to a single large leech. Similar observations were made on numerous other occasions during the periods of most active circulation in the lake. Such material never was found in the baskets except during autumnal and vernal overturns, and more was usually found in fall than in spring. It seems entirely reasonable to suppose that currents which would transport this heterogeneous collection of shoreward debris, and at least assist the scattering of littoral organisms, might also have a part in the autumnal dispersal of profundal bottom fauna.

Many instances indicating the effect of fall overturns and relief from stagnation could be cited from the data. Comparing the data presented in Figs. 59-61 with such variations as were shown by Protenthes larvae between October 10 and November 10, 1928 (Table XXVIII) or with that shown by Chironomus larvae in the 9-10 meter depth for various seasons (Table XXIV), will serve to illustrate what is meant. However, probably the most convincing evidence relative to the effect of overturns and stagnation is presented when the unanimity in general trend of variations, both qualitative and quantitative, for all depths and all seasons, is considered in the light of the whole seasonal cycle of physical-chemical factors.

Available evidence (p. 253) suggests the possibility that Kirkville Green Lake may never have a complete circulation of its profundal waters. The data for April 15, 1925 (Fig. 6), suggests that the lake was nearly homothermous throughout some time earlier in the same month, but its protection from wind is so complete and the slope of its bottom is so steep that the whole central

basin may, very possibly, never be completely stirred. If such is the case, it would account for the foul odor and character of the profundal mud. No bottom animals were found beneath the deeper water regions (Table XXXI) and this might also be explained on the basis of extreme and continuous stagnation.

In an attempt to test the effects of conditions imposed on profundal bottom animals by environmental factors of the profundal benthic habitat (partic-

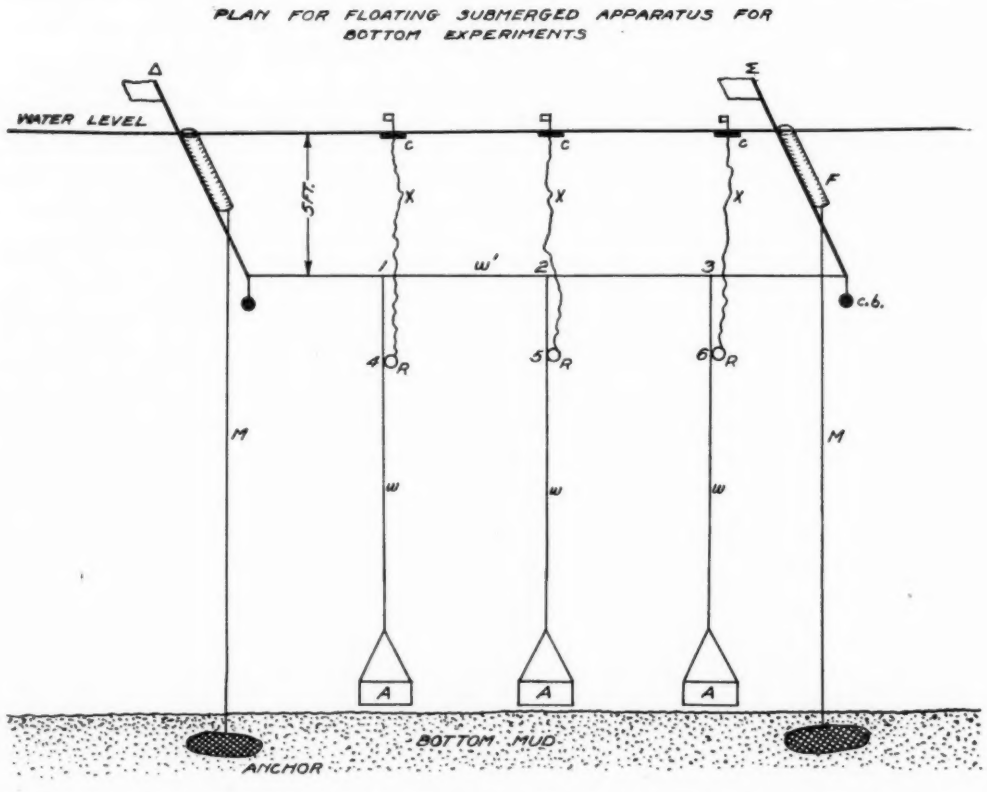


FIG. 62. Diagrammatic sketch of apparatus used to suspend experimental containers in lake at any desired level.

ABBREVIATIONS USED

A—wire-mesh basket for holding glass experimental containers. c—small cork floats. c.b.—counter balance. F—log float. M—tarred, ½-inch manila anchor rope. R—copper ring. w—paraffined No. 7 window sash cord. w^1—paraffined No. 8 window sash cord. X—paraffined No. 6 window sash cord. Δ and Σ—white buoy flags.

ularly during pronounced stagnation), several series of experiments were conducted on the lake bottom, in Douglas and Third Sister Lakes. These experiments were amplified and supplemented by others conducted in the laboratory. Only a brief statement of the aim and of the major features of the methods together with the principal results will be presented in this paper.

Series A. The object of this series of experiments was to determine whether or not profundal bottom animals could endure indefinitely conditions imposed by severe summer stagnation. The method employed was to place a

known number of specimens, usually 100, in 2-quart, glass, fruit jars; fill the jars with water; and in some instances add a little bottom mud; place the numbered jars in baskets constructed of galvanized wire netting; and suspend them at the desired level in the lake by means of equipment shown in Fig. 62. Some of the jars were carefully filled with oxygen-free water collected just above the bottom within the hypolimnion when stagnation conditions prevailed. Filling was done by introducing a long tube from the water sampler outlet to the bottom of the jar which was then overflowed for some time after it was full. Samples of the water used were analyzed for pH, dissolved oxygen, free carbon dioxide, and, in some instances, carbonates and bicarbonates, at the time of filling and at the close of the experiments. Temperature was also recorded at the same times. A little profundal mud which had been passed through a fine screen was added and into these jars were counted, from material freshly collected from deep water, a number of individuals of the different species of bottom animals. Usually representatives of but one genus were placed in a jar. Some of these containers were sealed in such a way as to include no air bubble; others were covered with a cap of fine-mesh, cloth netting. These jars, both open and closed, were lowered in a basket to the bottom where stagnation conditions were known to occur.

Controls were provided by placing in duplicate jars equal numbers of the same forms taken from the same collection. These control jars were filled with surface water. Profundal bottom mud was added to some; mud from the sublittoral to others; and still others contained no mud. In some of the experiments of this series, the control jars were filled to overflowing with surface water and sealed with no air bubble included; other jars were filled about one-half full of surface water and then sealed with a large air space; and still others were covered with the same kind of cloth netting used on the jars in the hypolimnion, and thus left open to the water. These controls were then lowered to a lake level known to be above the thermocline and in well aerated water. Such experiments were conducted in Douglas Lake during the summers of 1926, 1927 and 1928 and in Third Sister Lake during the summers of 1927 and 1928.

No two among the different species showed the same degree of resistance to the conditions imposed by stagnation. There was also a wide range of individual variation in resistance. In every experiment conducted, however, if it was continued long enough and under conditions which were severe enough, all Corethra, Chironomus and Protenthes larvae died. In no experiment did all *Tubificidae* or Pisidium die, although in two instances about 80 per cent died during an exposure of a little less than three months at 18 m. in Third Sister Lake during June, July and August. In all experiments, some individuals died in each jar, both experimental and control. This may have been caused by handling when they were placed in the jars, or those individuals might have been about to die from some other cause when collected.

In all instances, however, more individuals, usually many more, died in jars which were filled with water from the stagnation zone than in control jars. Results were not consistent in regard to which species of bottom animals showed the first deaths in experimental jars. Usually, however, all Corethra larvae were dead before all Chironomus or Protenthes larvae had succumbed. The last insect larvae to die usually were individuals of the genus Chironomus. In general, a larger percentage of *Tubificidae* survived than of any other type used.

Series B. These experiments were conducted with the hope that something might be learned concerning relative importance to profundal benthic animals of (1) the chemical nature of superimposed stagnant water and (2) the physical factors such as pressure, darkness, and low temperatures. Methods employed were identical with those described under Series A, except that (1) experimental jars lowered to the bottom within the hypolimnion were all sealed shut; (2) some contained surface lake water; (3) others, well water having no dissolved oxygen, 1-2 p.p.m. free carbon dioxide, and a pH of 7.3-7.6; and (4) still others, water from just above the bottom in the hypolimnion. The same species of experimental animals were used as in Series A.

All the animals lived longer in jars containing surface lake water than in either of the other sets. Many animals were alive in surface water jars at the close of the experiments. The animals died in the jars containing bottom lake water (O_2, 0.0. cc. per 1.; CO_2, 3.0 p.p.m.; pH, 7.0) more quickly than in the well water in which the dissolved oxygen, free carbon dioxide, and pH values were all somewhat similar.

Series C. The object of these experiments was to study the effect of stagnation conditions on activity of bottom animals. Jars were filled with water from just above the bottom within the hypolimnion in such a manner that no air bubble was included and otherwise treated as in Series A. The jars rested on the lake floor beneath the deeper hypolimnion. Observations regarding activity of the animals were usually made twice daily in Douglas Lake experiments, but much less frequently in Third Sister Lake experiments. Results in both lakes were uniformly similar. Corethra larvae and pupae remained active for a few days. Some larvae occupied various levels of the water in the jars and others, at times, burrowed into the mud. All Corethra larvae died if the experiments were continued for a few weeks. Sometimes they all died within one week. Chironomus larvae often constructed tubes in the mud and remained quite active for periods varying from 4 to 10 days. Usually most Chironomus larvae had become inactive within 5 days and were dead within 2 weeks. Results with Protenthes larvae were much the same as with Chironomus larvae. Individuals of Pisidium ceased movements within a few days and, with valves tightly closed, remained inactive thereafter. Some of these sphaeriids died within 2 weeks in experimental jars, others lived for months. In one instance, such a jar was set up with bottom water on October

11; 10 specimens of Pisidium introduced; the jar sealed; lowered to the lake bottom; and not opened until the following May 23. When this jar was opened, 2 empty shells were recovered and 2 inactive animals were found, which, however, appeared to be alive. When placed in surface lake water, these 2 specimens opened their valves, thrust out their feet and became quite active. No trace of the remaining 6 animals or their shells was found although the mud was carefully passed through a fine mesh screen. Specimens of Limnodrilus remained somewhat active throughout experiments of shorter duration and many individuals survived even the longer exposures. Activity of these forms usually consisted of slowly crawling through or on the mud. On a few occasions, specimens were seen to wave one end of the body slowly in the water while the other end remained within a burrow in the mud.

Series D. In this series of experiments, the effect of stagnation conditions on reproduction and life histories of profundal bottom fauna was studied. The general methods were those described under Series A. Experiments with Corethra eggs and with adult specimens of Limnodrilus and of Pisidium were conducted within the hypolimnion (South Fish-Tail depression, Douglas Lake) during the summer stagnation periods. Other experiments, using Limnodrilus cocoons and adults, and Pisidium adults were performed within the profundal zone, Third Sister Lake.

In addition to the lake-bottom experiments, certain others were conducted in the laboratory using tubificid cocoons. Cocoons were placed in bottles filled with water having no dissolved oxygen, 1.0-2.5 p.p.m. of free carbon dioxide, and a pH of 7.2-7.6. Some of these bottles were kept cold (3.0-10.0 °C.) others were kept at warmer temperatures (15.0-25.0 °C).

Controls for the experiments on Corethra eggs and tubificid cocoons were provided in three ways at different times. In some instances, controls consisted of eggs placed in open dishes filled with surface lake water and kept on a laboratory table; at other times, the control containers were large aquaria supplied with running surface lake water; and the third type of control was provided by placing eggs in jars filled with surface lake water and suspended within the epilimnion of the open lake.

Tubificid eggs hatched within 14-20 days after collection when kept in oxygenated water. They hatched a few days earlier in warm water (15-25°C.) than in cold water (4-10°C.). Under conditions closely resembling those of the hypolimnion during severe stagnation, some tubificid eggs developed, although not as quickly as did those in the control dishes. Not as large a percentage of the eggs hatched in experimental bottles as in the open control dishes.

Corethra eggs hatched more quickly when there was an abundance of dissolved oxygen in the water and temperature varied between 18 and 25°C. than they did when either temperature or oxygen content of the water was low. Elapsed time between laying and hatching of eggs varied between 2.5

and 13 days. No Corethra eggs hatched when subjected to conditions typical of deeper profundal regions during summer stagnation. Some Corethra eggs hatched (10-13 days after ovoposition) when placed in experimental jars which were covered with fine mesh grit-gauze and then lowered to the lake floor at a level well above the upper limit of the hypolimnion.

Experiments using mature specimens of Limnodrilus and of Pisidium were conducted in Douglas Lake during the summer and during all seasons of the year in Third Sister Lake; yet no positive evidence of reproduction of either of these organisms within experimental jars kept in the deeper profundal region was obtained.

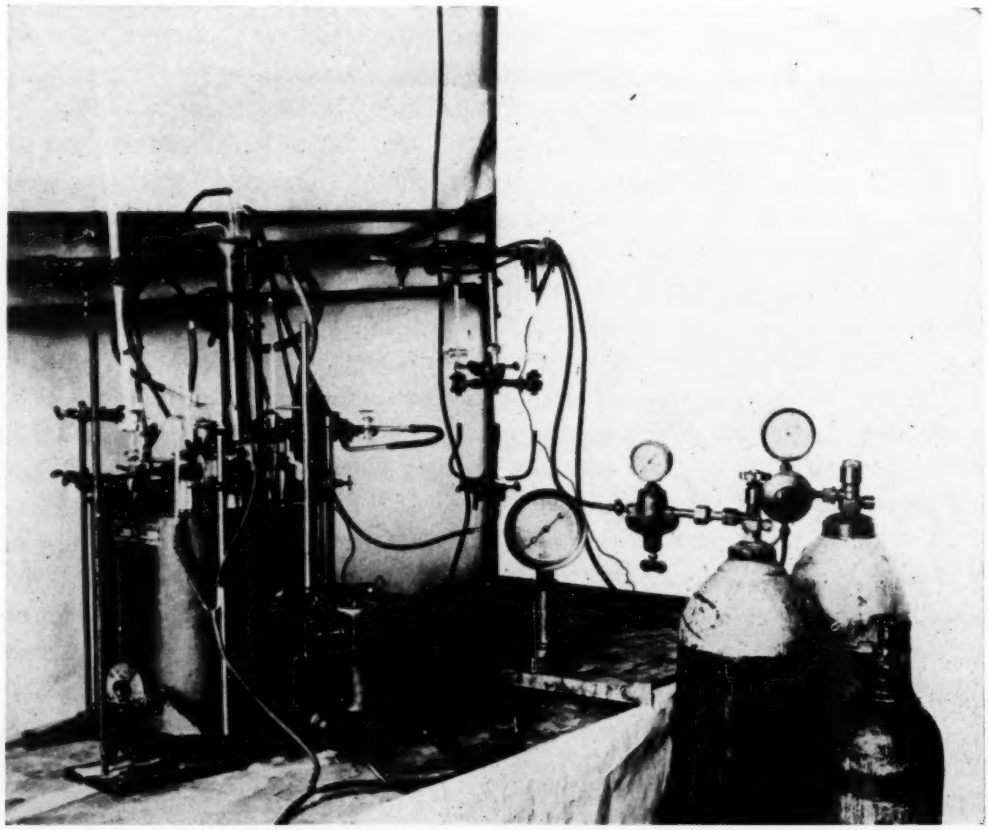


FIG. 63. Apparatus used in determining toleration ranges of profundal bottom organisms. Experimental *Series E*.

Series E. This set of experiments was conducted in the laboratory. The aim was to study toleration ranges of various benthic animals to environmental factors such as temperature, pH, free carbon dioxide, and dissolved oxygen. The apparatus used is shown in Fig. 63. High temperatures (25-50°C.) were obtained by a DeKotinsky Constant Temperature Apparatus; low temperatures by a circulating brine system, and pH was varied by introducing dilute HCL or KOH from a dropping burette into the water supply.

Dissolved oxygen, and free carbon dioxide content of the water were controlled by forcing various mixtures of oxygen, carbon dioxide and hydrogen into the water as it trickled down through a large tube filled with glass beads. Corethra, Chironomus and Protenthes larvae and, in a few instances, tubificids were used. The major portion of the experiments, however, concerned Corethra and Chironomus larvae.

None of the animals showed any sign of harm from low temperatures, even when kept in water just above the freezing point. Low oxygen content, although it always reduced activity, never resulted in a mortality significantly greater than that in control containers. This may have been due to the fact that none of these experiments were continued more than fifteen days. It is possible that longer exposure to oxygen-free water would have killed more individuals. Results of a long series of experiments using high temperatures may be summarized as follows: Corethra larvae were killed instantly by a temperature of 48°C. and died in 5 seconds at 47°C.; 2 minutes at 46°C.; 3-4 minutes at 45°C.; 40-60 minutes at 40°C.; 24-36 hours at 30°C.; but were still alive at the end of 15 days in water which varied between 20 and 25°C. Chironomus larvae were killed instantly by a temperature of 49°C.; died in 5-10 seconds at 48°C.; 40-50 seconds at 47°C.; 4-5 minutes at 45°C.; 40-60 minutes at 40°C.; 40-45 hours at 35°C.; 48-96 hours at 30°C.; but were alive and apparently normal after an exposure of 15 days to a temperature of 20-27°C.

Both Corethra and Chironomus larvae were rendered inactive within 3-5 minutes by exposures to a free carbon dioxide content of 800-900 p.p.m.; but all recovered later after exposures lasting up to 2 hours. Such experimental animals usually died, however, within 6-10 days after being subjected to these high concentrations of free carbon dioxide.

After some preliminary experiments, using certain organic acids which appeared to have a harmful effect aside from that due to the free hydrogen ion, pH was varied by using diluted solutions of hydrochloric acid or potassium hydroxide. Corethra larvae lived from 10-12 days in water having a pH of 3.0. When the pH was 6.0, 70 per cent were still alive after a 15 day exposure, but with the pH at 9.0-9.2, the larvae began dying within 3 days and all died within 14 days.

Biological Factors

Certain factors which affect life on the lake bottoms are specifically biological in their nature. It is true that these are, like others, affected by the annual physical-chemical cycle also. But despite their being influenced by such environmental conditions as temperature and chemical content of the water, they must be considered apart from such influences.

One of the most important factors influencing the quality and quantity of profundal, bottom animal life is insect transformation and emergence.

There can be no doubt that the enormous autumnal change within the deeper profundal is, in part, an actual shifting of the population, including the insect larvae, downward from the summer concentration zone. The data also indicate that a reciprocal swing in the opposite direction from deep to shallow water in the spring may be partly due to actual movement of the winter population, although any such shifting is of much less magnitude than in late autumn. But the most effective factor in the spring and early summer qualitative-quantitative reduction among the benthic fauna, and especially within the deeper profundal, is the emergence of great swarms of Chironomus and Corethra together with the less abundant Protenthes. In both Third Sister and Douglas Lakes, the period of marked quantitative decline among the insect larvae of the profundal benthic fauna is coincident with the most active emergence periods. The tables and figures show many examples, but a single instance will serve to illustrate the significance and magnitude of this factor. Reference to data for February 22, April 11 and June 6, 1928 (Table XXVIII), showing depth distributions of benthic animals, will reveal the effect of emergence upon that part of the bottom population which was composed of insect larvae. If these findings are compared with the "Tubificidae" column, the contrast between the effect upon total benthic population produced by the attainment of sexual maturity in those species which emerge and the effect of the same phenomenon on a species which spends all its life on the bottom will at once be apparent.

Although emergence of insects has the direct and immediate effect of bringing about great reduction in total population per sq. m., it leads to replenishment of this same population through egg laying and hatching of the next larval generation. Repeated observation on Douglas and Third Sister Lakes has convinced the author that Corethra, Chironomus and Protenthes, ovoposite promiscously all over the lake surface. There may, very possibly, be more eggs laid on shoreward waters but the writer has frequently seen females ovopositing on the lake surface over the center of South Fish-Tail depression, Douglas Lake. It is well known that the eggs sink soon after deposition and thus they must reach the lake floor in deep as well as shallow water. Juday (1921, p. 274) demonstrated that mud from the profundal zone of Lake Mendota contained Corethra eggs. The present writer was able to confirm this finding on several occasions with mud from the profundal zone in Douglas and Third Sister Lakes. In addition, young Chironomus larvae appeared in two such experiments at Douglas Lake and once when profundal mud from Third Sister Lake was being used. Other experiments performed by the author (p. 293) seem to indicate that these insect eggs do not hatch within the profundal zone where the temperature is low and the water chemically stagnant during the summer, but that those eggs which reach the bottom within the littoral and sublittoral do hatch during the summer. This would tend to build up the concentration zone in the region

above that of the most extreme stagnation. It is also possible that when the fall overturn sets in those eggs which were deposited within the profundal zone (particularly those laid by the later broods) may hatch. If so, these larvae, together with those which hatched in the shallower waters during late summer, would account for the increased total population for the whole lake in the late fall. Sharp autumnal decline of insect emergence; possible hatching of insect eggs beneath the deeper waters when stagnation is relieved; definite obliteration of the shallow water concentration zone and shifting of its massed population downward along the sloping lake floor, exemplify the interplay of various environmental factors which brings about the striking quantitative variations and depth distribution changes characteristic of the profundal benthic fauna when the fall overturn is in progress.

The tubificids in Douglas Lake were rarely found sexually mature during the summer. In October, 1928, however, many sexually mature specimens and a few cocoons were collected in bottom samples. Third Sister Lake yielded many sexually mature specimens and some cocoons each year in November. However, from February to the middle of April, each year, was the period when the largest proportion of sexually mature worms was collected and also during that period the greatest numbers of cocoons were taken. Following the appearance of the cocoons there was always a great increase in the total numbers of tubificids and many of these worms were small and immature. These periods of reproduction correspond with and explain their large numerical increases in Third Sister Lake. It seems probable that the greatest effect of the overturns on the *Tubificidae* is an acceleration of sexual maturity and activity, since these periods of circulation coincide roughly with the periods of maximum occurrence of cocoons and since the experiments described previously appear to show that some tubificid cocoons may develop even during stagnation within the profundal zone.

The possibility that the numbers of Corethra larvae swimming in the open water of the lake might be large enough to materially reduce the population left in the bottom mud was considered early in this investigation. Juday (1921) has shown that in Lake Mendota there is a considerable migration of full-grown Corethra larvae from bottom mud into the water during the night, but that young larvae occupy bottom water instead of the mud during the day; also that young larvae, "were found regularly in the net catches from the latter part of June to the first week in October."

The author made vertical hauls with a closing plankton net on many occasions during the course of this investigation, both in Douglas and in Third Sister Lakes. Only rarely were Corethra larvae of any age taken in Douglas Lake above depths of 10 meters in the daytime during June, July, and August. In Third Sister Lake, conditions with respect to presence of Corethra larvae in the water were quite different and there was a marked lack of consistency in the way larvae were distributed in the water on differ-

ent occasions. Usually they were present in the water up to a depth of 5 m. during the day. On certain occasions, they were found occupying the water at all levels from bottom to the surface, while at other times they were not found above the 15 m. level during the day. The whole question of diurnal migration of these larvae, in both lakes, is receiving further study and the results will be presented in a later paper.

SUMMARY

1. Physiography of the lakes is discussed; a hydrographic map of Third Sister Lake is given; and certain morphometric data relating principally to Douglas and Third Sister Lakes, are presented.

2. The extent and characteristics of the three benthic zones, littoral, sublittoral and profundal, in certain lakes, were investigated.

3. Douglas and Third Sister Lakes were found to have profundal zones of considerable extent and the profundal benthic habitat in each was studied in relation to (1) bottom deposits and (2) physical-chemical conditions of the superimposed water, as revealed by more than 180 vertical series of thermal-chemical determinations.

4. Qualitative-quantitative counts of bottom animals, based on a total of 1879 bottom samples from three lakes, showed the presence of a typical profundal benthic fauna in two of the lakes.

5. The organisms collected on the bottom form two ecological groups: (1) a typical profundal bottom fauna, comprising representatives of the genera Corethra, Chironomus, Protenthes, Limnodrilus, Pisidium, Musculium, and Hydromermis; and (2) a heterogeneous group of benthic types in which the Insecta were represented by 7 species; the Pelecypoda by 10; Hirudinea, 2; Hydracarina and Gastropoda, 13 each; and the Ostracoda and Nematoda, 1 species each.

6. Seasonal variation of *the typical profundal bottom fauna within the true profundal zone* showed the following general features for Douglas and Third Sister Lakes:

(a) Corethra larvae, always present in both lakes, were the most abundant inhabitants of the profundal zone, attaining a maximum population of more than 70,000 per sq. m.

(b) Limnodrilus was always present in Third Sister Lake and usually present in Douglas Lake.

(c) At certain times of the year in both lakes, all other typical profundal types were apparently absent except perhaps as eggs.

(d) Fluctuations of total profundal bottom fauna showed a marked periodicity with annual mid-summer minima and mid-winter maxima.

(e) Following the mid-summer minimum there was a slight and very slow increase in total population until late fall, when a very sudden and very large increase took place in the deepest regions of the lake.

(f) The decline leading to the mid-summer minimum began in early spring.

7. Data obtained from sampling series demonstrated that for profundal bottom animals the two major aspects of distribution are zonal and seasonal. The principal results may be summarized as follows:

(a) The distribution was not uniform from deep to shallow water.

(b) A concentration zone occurred in the upper profundal and lower sublittoral regions during the summer and the total population per unit area of bottom declined sharply above and below this zone.

(c) This concentration zone shifted downward into deeper water in the fall; occupied the deepest profundal during the winter; and then shifted up toward the summer level during the spring.

(d) The number of species and the number of individuals per unit area varied with the season, differently at each different depth, and in Douglas Lake, differently in each different depression.

(e) The number and kind of species and the number of individuals per unit area varied greatly in different lakes and in different parts of the same lake.

8. Ecological factors found to influence qualitative-quantitative variations and distribution were (a) bottom characteristics; (b) physical-chemical nature of the superimposed water; and (c) certain biological phenomena.

9. Both the densest and some of the most scanty populations were found on muddy bottoms. Complete absence of bottom animals occurred on (a) bottoms of clean sand and (b) muddy bottoms which had become very foul.

10. Chemical stagnation in the hypolimnion was found to be an important factor in the ecology of the profundal benthic fauna.

11. The semi-annual overturns produced very pronounced changes in the benthic population of the deeper profundal zone.

12. Emergence and egg laying of insects, variations in sexual activity of other benthic types, and the rate and time of hatching of eggs on the lake floor are all influenced by the physical-chemical seasonal cycle and, in turn, greatly affect the qualitative-quantitative variations of the profundal benthic population.

13. The profundal benthic fauna forms a very distinct ecological association, qualitatively limited by the severity of the habitat, but becoming quantitatively very rich at certain seasons.

14. It appears probable that the profundal benthic association has been derived from the sublittoral fauna and this in turn from the fauna of the littoral zone.

15. Considering the interplay of physical, chemical, and biological factors of the environment, it seems evident that the pronounced effectiveness of the physical-chemical stagnation is due not to the variations of any one factor in

the complex, but rather to the combined action of variations of each factor in the presence of variations of the others.

16. Evidence now available indicates that the members of the profundal benthic fauna are facultative rather than obligatory "anaerobes" and that they endure rather than select an anaerobic environment.

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EXPLANATION OF TABLES V TO XXXIV

In the tables showing variations of physical factors, temperatures are expressed in degrees Centigrade; depths in meters; dissolved oxygen in cubic centimeters per liter; free carbon dioxide in parts per million; and methyl orange alkalinity in parts per million of calcium carbonate.

Abbreviations: *S.*, surface; *u.l.H.*, upper limit of the hypolimnion; *B.*, bottom; *N.S.*, no stratification; *cc. per l.*, cubic centimeters per liter; *p.p.m.*, parts per million; *M.O.*, methyl orange; *pH*, hydrogen ion concentration; *Cor.*, Corethra; *Chir.*, Chironomus; *Prot.*, Protenthes; *Tub.*, Tubificidae; *Suc.*, Succinea; *Phy.*, Physa; *Val.*, Valvata; *Gon.*, Goniobasis; *Plan.*, Planorbis. Other abbreviations used are all too well known to need explanation.

TABLE V. *Analysis of bottom mud.*

Station No.	Depression	Depth meters	Weight, in grams, of				Wet Mud		Dry Mud	
			Wet Mud	Dry Mud	Residue after ignition	Loss on ignition	% water	% solid matter	% non-volatile matter	% volatile matter
1	South Fish-Tail...	24	1030.7	84.52	56.59	27.93	91.8	8.2	67.0	33.0
30	Fairy Island.....	28	1042.6	86.54	58.05	28.49	91.7	8.3	67.1	32.9
10	Grapevine Point...	25	1044.2	108.6	74.76	33.84	89.6	10.4	68.8	31.2
50	Robert's Point....	22	1051.15	118.78	82.55	36.23	88.7	11.3	69.5	30.5
20	Sedge Point.....	24	1053.04	120.8	85.77	35.03	88.5	11.5	71.0	29.0
40	Stony Point.....	19	1058.45	150.3	114.2	36.07	85.8	14.2	76.0	24.0
(1a)	Third Sister Lake .	18	1582.3	857.4	792.7	67.70	45.8	54.2	92.1	7.9
(1b)	" " "	18	1062.4	201.2	129.7	71.50	81.1	18.9	64.5	35.5
(1c)	" " "	18	2113.0	1519.5	1456.0	63.50	28.1	71.9	95.9	4.1

Samples from each of the 6 major depressions of Douglas Lake and from the 18 m. depth of Third Sister Lake.
Volume of mud = 1000 cc. in each sample.
1a—Bottom mud as it occurs in the lake, *i.e.* alternate layers of 1b and 1c (mixed together in this sample).
1b—A sample of the black organic detritus layers.
1c—A sample of the gray clay layers.

TABLE VI. *Seasonal variation of physical-chemical factors.*
South Fish-Tail depression—Douglas Lake.

Station 1 — *23 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923																	
July 5	22.1	10.7	8.5	12–15	7.1	8.4	7.4	7.3	4.26	2.9	0.28						
9	22.2	9.7	8.3	11–15	8.8	8.4	7.6	7.2									
13	23.9	11.5	8.5	11–15	7.9	8.4	7.6	7.2	5.1	2.1	0.08						
19	23.7	11.5	8.0	11–15	7.8	8.4	7.4	7.2	5.27	2.51	0.70						
24	22.8	10.2	8.5	9–15	11.4	8.4	7.3	7.1	3.49	0.84	0.06	0.0	5.0	15.0	117	118	126
27	21.1	10.8	8.4	9–14	10.2	8.2	7.2	7.1	4.54	1.93	0.0	0.0	1.0	12.0			
31	21.7	10.0	8.3	9–15	10.0	8.2	7.2	7.1	4.18	2.93	0.0	0.0	6.0	9.5	119	120	130
Aug. 6	22.5	9.8	8.3	11–15	9.7	8.2	7.2	7.1	6.07	1.60	0.0	0.0	7.0	16.0	120	127	136
16	21.8	9.4	8.8	11–15	10.4	8.1	7.2	7.1	6.21	0.97	0.0	0.0	10.0	19.0	124	128	135
1926																	
June 22	17.2	6.9	6.8	16–18	6.7	8.4	7.8	7.3									
July 1	19.0	9.8	6.5	15–18	6.0	8.4	7.3	7.2	6.05	0.61	0.0						
13	18.7	9.6	6.9	15–20	9.9	8.4	7.2	7.2	6.16	0.00	0.0	0.0	15.0	19.0	126	129	130
22	22.3	9.9	7.3	14–18	8.1	8.4	7.2	7.2	3.52	0.66	0.0	0.0	5.0	16.0	124	130	136
Aug. 9	22.7	9.7	7.6	15–19	7.1	8.5	7.2	7.1	6.28	0.0	0.0	0.0	10.0	13.0	125	129	131
13	21.0	9.1	7.7	14–19	9.2	8.5	7.2	7.1	5.88	0.0	0.0	0.0	11.0	16.0	129	140	142
26	19.3	10.1	8.2	15–19	7.9	8.5	7.2	7.0	6.77	0.0	0.0	0.0	11.0	19.0	125	130	138
1927																	
June 24	18.4	16.6	11.7	8–9	1.0												
July 2	23.4	19.9	10.7	6–8	2.9	8.4	8.3	7.3	5.58	5.44	0.0	0.0	0.0	8.0	118	115	123
6	20.2	13.2	11.0	12–15	5.4	8.4	7.6	7.3	6.00	2.86	0.14	0.0	3.0	9.0	117	122	124
27	21.2	11.5	10.9	12–18	8.0	8.4	7.1	7.1	5.58	0.00	0.0	0.0	8.0	13.0	117	123	127
Aug. 15	20.4	11.5	11.0	16–20	6.7	8.4	7.1	7.0	6.14	0.00	0.0	0.0	11.0	13.0	114	123	123
1928																	
June 22	19.9	10.4	9.0	13–19	5.6	8.4	7.1	7.1									
27	17.6	11.1	9.4	15–17	3.3	8.4	7.1	7.0	5.37	1.60	0.0	0.0	5.0	11.0	114	125	126
July 4	20.9	11.3	9.6	13–17	5.7	8.2	7.2	6.9	5.40	0.20	0.0	0.0	7.0	12.0	118	125	127
21	21.7	12.2	9.9	11–17	8.3	8.0	7.1	6.9	5.38	0.19	0.0	0.0	7.0	15.0	111	122	124
Aug. 12	23.2	11.8	10.6	13–18	8.3	8.2	7.0	6.9	5.44	0.03	0.0	0.0	9.0	12.0	115	123	126
14	24.2	11.6	10.6	13–18	8.5	8.2	7.0	6.8	....	0.0	0.0	0.0	9.0	12.0	...	123	125
Oct. 20	11.4	N.S.	10.9	N.S.	N.S.	7.8	N.S.	7.8	7.24	N.S.	6.34	1.5	N.S.	2.0	180	N.S.	152

TABLE VII. *Seasonal variation of physical-chemical factors.*
Grapevine Point depression—Douglas Lake.

Station 10 *24 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923																	
Aug. 18	20.8	11.0	9.4	12–18	9.4	8.4	7.1	7.0									
1926																	
July 14	19.5	N.S.	18.3	N.S.	N.S.	8.4	N.S.	8.2	6.10	N.S.	5.06	0.0	N.S.	0.0	125	N.S.	129
24	20.1	N.S.	18.3	N.S.	N.S.	8.4	N.S.	7.6	6.07	N.S.	2.96	0.0	N.S.	4.0	123	N.S.	132
29	23.2	N.S.	18.0	N.S.	N.S.	8.4	N.S.	7.3	6.35	N.S.	0.55	0.0	N.S.	12.0	130	N.S.	130
Aug. 6	22.2	N.S.	17.8	N.S.	N.S.	8.4	N.S.	7.3	6.09	N.S.	0.28	0.0	N.S.	12.0	125	N.S.	133
23	19.8	N.S.	18.1	N.S.	N.S.	8.5	N.S.	8.2	6.0	N.S.	5.28	0.0	N.S.	0.0	125	N.S.	129
1927																	
July 8	19.8	16.6	15.2	15–16	1.2	8.4	7.6	7.4	5.72	3.55	1.53	0.0	5.0	8.0	110	116	123
26	20.5	16.0	15.1	18–21	3.2	8.4	7.3	7.2	5.58	0.0	0.0	0.0	10.0	11.0	112	122	123
Aug. 12	20.0	N.S.	17.8	N.S.	N.S.	8.4	N.S.	7.8	5.44	N.S.	3.90	0.0	N.S.	5.0	113	N.S.	121
1928																	
June 26	17.6	N.S.	15.6	N.S.	N.S.	8.4	N.S.	7.5									
28	18.0	N.S.	15.9	N.S.	N.S.	8.4	N.S.	7.4	5.79	N.S.	3.49	0.0	N.S.	6.0	112	N.S.	128
July 1	19.4	N.S.	16.0	N.S.	N.S.	8.2	N.S.	7.2	5.72	N.S.	3.35	0.0	N.S.	8.0	111	N.S.	127
24	22.0	18.0	16.5	12–14	3.0	8.1	7.4	7.1	5.00	1.52	0.34	0.0	4.0	9.0	114	120	123
Aug. 7	21.8	N.S.	16.5	N.S.	N.S.	8.2	N.S.	7.2	5.56	N.S.	0.0	0.0	N.S.	10.0	117	N.S.	126
14	22.8	N.S.	16.4	N.S.	N.S.	8.2	N.S.	7.2	5.35	N.S.	0.0	0.0	N.S.	8.0	114	N.S.	126

TABLE VIII. *Seasonal variation of physical-chemical factors. Sedge Point depression—Douglas Lake.*

Station 20 *25 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923																	
Aug. 18	20.4	12.5	10.6	12–17	7.4	8.1	7.1	7.1	5.72	0.07	0.0						
1926																	
July 15	19.9	N.S.	16.3	N.S.	N.S.	8.5	N.S.	7.2	5.91	N.S.	0.0						
16	22.0	N.S.	17.2	N.S.	N.S.	8.4	N.S.	7.2	6.27	N.S.	0.0	0.0	N.S.	12.0	120	N.S.	131
Aug. 5	22.7	N.S.	17.2	N.S.	N.S.	8.5	N.S.	7.3	5.30	N.S.	0.0	0.0	N.S.	16.0	126	N.S.	132
27	20.1	N.S.	16.7	N.S.	N.S.	8.6	N.S.	7.6	6.56	N.S.	3.28	0.0	N.S.	3.0	122	N.S.	125
1927																	
July 9	20.0	16.5	12.9	14–16	2.0	8.4	7.6	7.3	5.72	3.21	0.0	0.0	4.0	9.0	116	119	123
Aug. 5	20.1	14.1	13.1	18–20	5.0	8.4	7.4	7.2	5.72	0.0	0.0	0.0	5.0	12.0	118	123	123
15	19.9	14.8	14.3	21–22	3.0	8.4	7.3	7.3	5.86	?	0.0	?	?	26.0	115	122	122
1928																	
June 27	17.5	N.S.	12.2	N.S.	N.S.	8.4	N.S.	7.1	5.37	N.S.	0.0	0.0	N.S.	4.0	110	N.S.	127
July 7	22.1	N.S.	14.6	N.S.	N.S.	8.0	N.S.	7.1	5.72	N.S.	0.03	0.0	N.S.	9.0	118	N.S.	125
24	22.6	18.3	15.1	12–14	2.1	8.1	7.3	7.1	5.56	1.59	0.0	0.0	4.0	10.0	116	120	125
Aug. 9	22.6	17.0	14.6	15–17	3.0	8.2	7.5	7.1	5.58	2.09	0.0	0.0	5.0	13.0	115	122	125
14	24.4	16.4	15.2	16–18	3.2	8.2	7.2	7.0	...	1.18	0.0	0.0	7.0	11.0	...	123	126

TABLE IX. *Seasonal variation of physical-chemical factors.*
Fairy Island depression—Douglas Lake.

Station 30 *28 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923																	
Aug. 15	20.4	10.3	9.0	14–17	8.0	8.2	7.2	7.0	6.63	0.27	0.0						
1926																	
July 16	19.6	9.7	8.7	18–24	7.7	8.4	7.2	7.2	5.30	0.00	0.0						
28	22.8	13.0	12.0	19–22	3.8	8.4	7.4	7.2	6.48	0.20	0.0	0.0	10.0	16.0	126	131	135
Aug. 6	21.8	N.S.	11.3	N.S.	N.S.	8.4	N.S.	7.3	6.7	N.S.	0.0	0.0	N.S.	18.0	127	N.S.	136
1927																	
July 4	19.7	14.5	12.6	15–18	3.5	8.5	7.4	7.3	5.58	1.95	0.0	0.0	3.0	5.0	114	120	
16	23.4	15.3	12.6	16–18	2.3	8.4	7.4	7.3	5.44	1.67	0.0	0.0	7.0	11.0	113	119	127
Aug. 10	19.3	13.9	12.8	20–22	4.2	8.4	7.3	7.2	5.23	0.00	0.0	0.0	12.0	13.0	110	121	122
1928																	
June 26	17.6	12.2	9.8	21–23	2.6	8.4	7.1	7.0									
July 1*	19.6	11.8	10.6	20–23	4.2	8.2	7.0	7.0	5.72	0.00	0.0	0.0	10.5	13.0	110	123	123
23**	21.7	18.0	11.6	12–13	1.5	8.0	7.5	7.0	5.14	2.84	0.0	0.0	2.0	10.0	112	121	
Aug. 6	21.6	16.4	12.3	15–17	2.1	8.1	7.1	6.9	5.42	0.97	0.0	0.0	5.0	13.0	115	123	130
14***	23.5	16.4	12.5	14–17	3.0	...	7.2	7.0	...	0.00	0.0	...	7.0	12.0	...	124	126

*Values given under u.l.H. for pH, O_2, CO_2, and M.O. Alk. are those recorded at 25 meters, there being no determinations at exactly 23 meters, the exact upper limit of the hypolimnion.
**Values given under u.l.H. for pH, O_2, CO_2, and M.O. Alk. are those recorded at 15 meters.
***Values given under u.l.H. for pH, O_2, CO_2, and M.O. Alk. are those recorded at 20 meters.

TABLE X. *Seasonal variation of physical-chemical factors.*
Stony Point depression—Douglas Lake.

Station 40 *19 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1926																	
July 17	20.0	N.S.	18.0	N.S.	N.S.	8.4	N.S.	7.8	6.09	N.S.	4.95						
Aug. 16	21.2	N.S.	18.0	N.S.	N.S.	8.5	N.S.	7.4	6.28	N.S.	0.48	0.0	N.S.	10.0	125	N.S.	130
27	20.2	N.S.	18.0	N.S.	N.S.	8.6	N.S.	7.4	6.49	N.S.	0.06	0.0	N.S.	10.0	122	N.S.	129
1927																	
July 9	20.0	N.S.	16.0	N.S.	N.S.	8.4	N.S.	7.6	5.72	N.S.	3.07	0.0	N.S.	5.0	116	N.S.	120
25	20.8	N.S.	19.2	N.S.	N.S.	8.4	N.S.	7.8	4.88	N.S.	2.96	0.0	N.S.	4.0	111	N.S.	121
Aug. 15	19.9	N.S.	19.0	N.S.	N.S.	8.4	N.S.	8.0	5.79	N.S.	4.60	0.0	N.S.	0.5	117	N.S.	122
1928																	
June 28	18.1	N.S.	16.0	N.S.	N.S.	8.4	N.S.	7.3	5.58	N.S.	2.93	0.0	N.S.	9.0	113	N.S.	127
July 16	21.0	17.2	17.4	12–15	3.0	8.0	7.4	7.2	5.51	1.74	1.53	0.0	6.0	10.0	118	124	125
25	22.6	18.6	16.4	13–14	1.4	8.1	7.3	7.2	5.56	1.52	0.0	0.0	5.0	9.0	114	120	127
Aug. 7	22.5	N.S.	19.1	N.S.	N.S.	8.2	N.S.	7.5	5.62	N.S.	2.29	0.0	N.S.	5.0	117	N.S.	126
14	24.8	N.S.	17.8	N.S.	N.S.	8.2	N.S.	7.3	...	N.S.	0.34	0.0	N.S.	9.0	...	N.S.	125

TABLE XI. *Seasonal variation of physical-chemical factors.*
Roberts Point depression—Douglas Lake.

Station 50 *23 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1926																	
July 23	21.9	18.6	14.4	11–12	1.2	8.4	8.3	7.2	6.27	...	0.03	0.0	1.0	15.0	125	125	134
Aug. 3	24.4	N.S.	15.3	N.S.	N.S.	8.5	N.S.	7.2	6.28	N.S.	0.00	0.0	N.S.	16.0	125	N.S.	134
11	21.2	N.S.	14.2	N.S.	N.S.	8.6	N.S.	7.2	6.49	N.S.	0.00	0.0	N.S.	16.0	124	N.S.	132
16	21.5	N.S.	14.0	N.S.	N.S.	8.5	N.S.	7.2	6.35	N.S.	0.00	0.0	N.S.	16.0	124	N.S.	132
1927																	
July 5	20.0	13.7	12.2	14–16	4.3	8.4	7.5	7.3	5.72	2.51	0.83	0.0	4.0	6.0	113	122	123
18	21.0	13.5	12.5	14–17	5.4	8.4	7.4	7.3	5.30	1.18	0.69	0.0	7.0	10.0	114	122	124
Aug. 12	19.6	14.4	13.0	17–19	3.8	8.4	7.4	7.3	5.51	0.00	0.00	0.0	10.1	25.0	113	122	123
1928																	
June 26	17.5	13.4	13.7	17–18	1.4	8.4	7.3	7.2									
July 5	20.8	14.5	12.6	16–17	1.1	8.2	7.3	7.1	5.57	0.22	0.00	0.0	6.0	10.0	116	123	124
12	20.8	17.8	12.5	10–11	2.4	8.1	7.5	7.1	5.44	3.55	0.00	0.0	3.0	10.0	118	125	126
25	23.0	16.5	13.0	11–14	4.5	8.1	7.2	7.1	5.62	1.73	0.00	0.0	3.0	13.0	113	121	125
Aug. 6	22.4	13.9	13.0	13–18	5.6	8.1	7.1	7.0	5.49	0.00	0.00	0.0	9.0	12.0	114	121	124
14	23.7	15.1	13.2	14–16	3.2	8.1	7.3	7.0	....	0.00	0.00	0.0	5.0	11.0	...	123	125
Oct. 20	11.1	N.S.	11.1	N.S.	N.S.	7.8	N.S.	7.8	7.10	N.S.	6.48	1.5	N.S.	1.5	163	N.S.	156

TABLE XII. *Seasonal variation of physical-chemical factors. Third Sister Lake.*

Station 1 — *18 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923														
Feb. 17	0.1	...	5.0											
28	0.5	...	4.75											
Apr. 25	13.1	6.5	4.9	1–7	6.8	7.8	7.6	7.4						
May 2	14.9	7.6	4.7	3–7	6.5	8.4	7.8	7.4						
1926														
Oct. 19	12.7	...	...	....	...	7.4	...	...	6.1					
20	12.2	8.2	6.5	8–10	4.2	7.4	7.1	7.0	6.28	0.34	0.0	2.0	9.0	13.0
26	9.7	7.5	6.5	11–12	1.0	7.4	7.1	7.0	6.20	0.00	0.0	2.0	9.0	12.0
Nov. 2	8.1	N.S.	6.6	N.S.	N.S.	7.4	N.S.	7.0	6.1	N.S.	0.0	2.0	N.S.	12.0
10	5.6	N.S.	6.8	N.S.	N.S.	7.4	N.S.	7.4	5.72	N.S.	5.72	2.0	N.S.	3.5
17	6.5	N.S.	6.0	N.S.	N.S.	7.4	N.S.	7.4	5.75	N.S.	6.18	3.0	N.S.	3.5
24	3.8	N.S.	4.0	N.S.	N.S.	7.5	N.S.	7.5	5.81	N.S.	6.2	3.0	N.S.	3.0
1927														
Jan. 5	0.9	...	4.2	....	...	7.6	...	7.0	6.65	....	2.17	5.0	...	11.0
Apr. 16	8.0	N.S.	6.4	N.S.	N.S.	7.8	N.S.	7.4	7.24	N.S.	5.46	1.5	N.S.	8.0
June 1	18.2	8.0	6.9	5–10	7.6	8.0	7.2	7.1	6.53	4.73	0.00	1.0	7.0	13.0
9	18.4	8.1	6.8	4–10	8.1	7.9	7.3	7.0	6.38	5.32	0.00	1.5	5.5	12.5
Aug. 27	21.3	9.3	7.7	5–11	10.8	8.4	7.0	7.0	5.71	0.00	0.00	0.0	8.0	13.0
Sept. 13	23.6	9.0	7.3	5–9	11.2	8.4	7.0	6.9	5.31	0.76	0.00	0.0	8.0	10.0
15	24.1	9.1	7.3	5–9	10.9	8.4	7.0	7.0	5.37	0.06	0.00	0.0	7.0	10.0
16	24.5	9.2	7.4	5–9	10.8									
17	25.5	9.0	7.4	4–10	13.5	8.4	7.0	7.0	5.44	0.05	0.00	0.0	7.0	15.0
27	18.2	7.8	7.3	6–12	9.7	7.8	7.0	6.9	5.50	0.00	0.00	1.0	6.0	17.0
Oct. 4	18.0	8.8	7.2	6–10	8.5	7.8	7.0	6.9	5.45	0.00	0.00	1.5	5.0	18.0
11	15.8	9.1	7.2	7–10	6.2	7.7	7.0	6.8	5.58	0.00	0.00	2.2	5.0	18.0
18	12.8	8.3	7.2	9–11	4.2	7.6	7.0	6.8	5.79	0.13	0.00	3.0	5.0	20.0

TABLE XII. *(Continued)*

Station 1 — *Seasonal variation of physical-chemical factors. Third Sister Lake.* — *18 meters*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1927														
Oct. 25	13.0	8.3	7.3	9–11	4.1	7.7	7.0	6.8	5.86	0.13	0.0	3.0	6.0	22.0
Nov. 1	13.8	8.4	7.3	9–11	2.9	7.8	7.0	6.8	5.62	0.00	0.0	2.5	7.0	25.0
8	9.0	8.1	7.4	12–13	1.0	7.6	7.3	7.0	5.55	0.61	0.06	3.0	5.0	15.0
15	9.2	N.S.	7.4	N.S.	N.S.	7.6	N.S.	7.0	5.44	N.S.	0.06	3.0	N.S.	15.0
22	6.7	N.S.	6.6	N.S.	N.S.	7.4	N.S.	7.3	5.0	N.S.	4.61	5.0	N.S.	5.0
26	7.0	N.S.	6.8	N.S.	N.S.	7.5	N.S.	7.5	4.96	N.S.	4.76	5.0	N.S.	5.0
29	7.2	N.S.	7.2	N.S.	N.S.	7.6	N.S.	7.5	5.67	N.S.	5.3	4.0	N.S.	5.0
Dec. 6	4.6	N.S.	4.8	N.S.	N.S.	7.7	N.S.	7.6	5.56	N.S.	5.31	3.0	N.S.	4.0
1928														
Jan. 23	1.0	N.S.	2.8	N.S.	N.S.	8.0	N.S.	7.7	6.52	N.S.	4.43	2.0	N.S.	4.0
Feb. 22	0.5	...	3.0	...	...	7.6	...	7.4	5.73	...	3.69	3.0	...	5.0
Apr. 6	10.3	5.5	4.8	5–7	2.0	7.8	7.6	7.5	5.27	6.0	5.03			
20	8.6	N.S.	6.2	N.S.	N.S.	7.8	N.S.	7.7	4.05	N.S.	4.98			
May 23	20.0	10.1	6.5	1–6	9.9	8.0	7.8	7.7	4.36	6.57	2.21	0.5	1.0	11.0
June 6	15.0	8.3	6.5	5–9	6.3	8.0	7.4	7.0	4.99	3.59	0.12	2.0	3.0	10.0
Oct. 10	14.8	9.6	7.6	5–9	4.9	7.5	6.6	6.5	6.00	0.00	0.00	1.5	5.0	9.0
Nov. 3	11.4	8.0	7.6	11–12	1.3	7.3	6.7	6.5	5.65	0.50	0.00			
10	8.6	N.S.	7.7	N.S.	N.S.	7.5	N.S.	6.9	4.74	N.S.	0.01	3.0	N.S.	12.0
24	4.7	N.S.	4.6	N.S.	N.S.	7.3	N.S.	7.3	7.04	N.S.	3.76	1.0	N.S.	2.0
Dec. 15	1.4	N.S.	4.2	N.S.	N.S.	7.2	N.S.	7.2	8.45	N.S.	2.6	2.5	N.S.	3.0
1929														
Feb. 9	0.5	...	4.0	...	...	7.2	...	6.9	8.18	....	1.83	1.0	...	3.0

TABLE XIII. *Vertical temperature series. Green Lake—Kirkville, New York, 1925.*

Depth Meters	April 13	April 15	May 9	June 17	June 18	June 22	June 26	June 27	June 28
0.....	8.8	7.8	12.1	22.4	22.8	24.0	21.3	22.5	23.9
0.5....	...	...	....	....	....	....	21.1		
1.....	...	7.8	11.7	20.8	21.1	22.1	20.8		
2.....	...	7.8	11.7	20.7	20.8	21.0	20.5		
3.....	...	7.8	10.8	20.7	20.6	20.4	20.3		
4.....	...	6.7	10.6	18.9	19.0	19.3	19.3		
5.....	7.1	6.2	10.4	15.8	15.8	18.0	18.3		
6.....	...	6.1	...	14.2	14.1	16.6	17.3		
7.....	...	6.1	...	12.5	12.4	14.1	16.3		
8.....	...	6.1	...	11.7	11.6	12.8	15.6		
9.....	...	5.9	...	10.4	10.5	12.1	15.0		
10.....	5.8	5.9	9.1	9.5	9.6	11.1	14.9	14.8	14.9
11.....	...	5.7	7.9	...	8.9	10.2			
12.....	...	5.5	6.1	...	8.2	10.1	11.8		
13.....	...	5.2	5.4	...	7.5	8.5			
14.....	...	5.0	5.4	...	6.3	6.8	7.3		
15.....	4.8	4.8	4.8	5.6	5.6	5.6	5.6		
16.....	...	4.7	5.0	4.8	4.7	4.5	4.6		
17.....	...	4.7	5.6	5.7	4.8	5.1	5.1		
18.....	...	4.6	6.3	6.2	5.4	5.6	5.8		
19.....	...	4.4	6.6	7.0	6.1	...	6.7		
20.....	4.4	4.4	7.2	7.1	7.1	7.0	7.0	7.0	7.0
21.....	...	...	...	...	...	...	7.0		
22.....	...	...	...	...	...	...	7.0		
25.....	4.4	4.4	7.2	8.2	8.2	7.2	7.1		
30.....	4.7	4.7	7.2	7.9	8.1	7.4	7.7	7.3	7.3
35.....	4.8	4.8	7.2	7.9	8.1	7.5	7.4		
40.....	4.9	4.9	7.3	7.6	7.6	7.3	7.5	7.5	7.5
45.....	5.0	5.1	7.3	7.6	7.6	7.5	7.5		
50.....	5.0	5.1	7.4	7.7	7.8	7.8	7.6	7.6	7.7
55.....	5.0	5.2	...	7.8	7.8	8.0	7.8		
57.....	...	...	7.7						
58.....	...	...	...	7.9					
59.....	...	...	...	...	7.9	8.0	8.1		
59.5....	...	...	...	...	...	...	8.1		
60.....	5.1	5.3	...	...	...	...	...	8.1	8.1
61.....	...	5.6	...	...	...	...	...	...	8.2

Temperatures expressed in degrees Centigrade.

TABLE XIV. *Seasonal variation of bottom fauna. South Fish-Tail Depression—Douglas Lake.*

Station 1 *21-24 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1923							
July 5	10	267	31	0	0	0	298
11	30	149	26	0	0	0	175
16	12	93	34	0	0	0	127
20	10	103	58	0	0	0	161
21	13	86	41	0	0	0	127
Aug. 1	6	23	0	0	0	0	23
10	23	4	0	0	0	0	4
18	3	30	15	0	45	0	90
1926							
June 19	2	334	0	0	0	0	334
30	10	138	40	23	9	0	210
July 2	5	98	27	45	27	0	197
7	15	131	27	21	60	3	242
22	10	58	23	0	23	0	104
28	40	87	66	0	30	0	183
Aug. 9	10	5	0	5	14	0	24
10	10	5	9	0	5	0	19
11	5	6	8	0	4	0	18
13	10	45	14	5	9	0	73
26	10	147	0	0	54	0	201
1927							
June 24	10	187	36	32	23	0	278
July 2	5	178	27	27	9	0	241
20	35	55	16	8	8	0	87
25	10	49	5	5	14	0	73
28	25	38	15	0	2	0	55
Aug. 15	5	36	18	0	10	0	64
1928							
June 22	2	289	0	0	0	0	289
27	5	303	0	0	10	0	313
July 2	10	316	14	5	9	0	344
9	15	305	12	6	18	0	341
19	10	200	18	9	40	0	267
30	10	10	5	0	9	0	24
Aug. 2	5	27	18	0	9	0	54
12	15	39	3	0	18	0	60
Oct. 20	5	3,448	80	36	18	0	3,582
Total	401						

TABLE XV. *Seasonal variation of bottom fauna. Sedge Point depression—Douglas Lake.*
Station 20 *21-24 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1923							
Aug. 18	15	24	12	0	0	0	36
1926							
July 15	10	726	9	116	5	5	931
26	10	489	0	49	14	0	552
Aug. 5	25	4	0	0	10	0	14
27	15	274	0	9	15	0	298
1927							
July 9	10	1,098	58	23	32	0	1,211
23	10	698	49	32	40	0	819
Aug. 5	5	80	125	0	27	0	232
15	5	45	45	0	0	0	90
1928							
June 28	2	3,355	45	45	0	0	3,445
July 7	5	898	9	36	0	0	943
8	2	867	0	44	0	0	911
24	10	18	0	0	14	0	32
Aug. 9	10	63	14	0	23	0	100
Total	134						

TABLE XVI. *Seasonal variation of bottom fauna. Fairy Island depression—Douglas Lake.*
Station 30 *21-28 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1923							
Aug. 15	30	5	3	0	26	0	34
1926							
July 16	10	303	63	5	18	0	389
28	10	156	14	5	32	0	207
Aug. 6	15	69	63	3	72	0	207
1927							
July 4	10	94	27	0	54	0	175
16	10	40	27	5	45	0	117
Aug. 10	10	9	63	0	45	0	117
1928							
July 1	2	43	7	0	23	0	73
23	10	36	76	0	49	0	161
Aug. 6	5	27	18	0	45	0	90
Total	112						

TABLE XVII. *Seasonal variation of bottom fauna. Roberts Point depression—Douglas Lake.*

Station 50 *21-22 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
July 14	15	503	37	14	31	0	585
23	10	391	14	9	49	0	463
Aug. 3	10	58	18	0	23	0	99
11	12	23	0	0	49	0	72
16	10	63	18	23	67	0	171
1927							
July 5	5	196	356	151	0	0	703
18	5	169	400	89	0	0	658
Aug. 12	10	129	85	0	129	0	343
1928							
July 5	5	640	36	18	116	0	810
12	5	223	89	54	98	0	464
25	5	45	89	27	45	0	206
Aug. 6	25	63	36	18	11	8	136
10	46	151	42	11	126	10	340
Oct. 20	4	2,278	144	112	67	156	2,757
Total	167						

TABLE XVIII. *Seasonal variation of bottom fauna. Stony Point depression—Douglas Lake.*

Station 40 *17-19 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
July 17	10	67	14	254	63	174	572
Aug. 16	10	54	18	14	63	49	198
27	5	738	18	63	89	45	953
1927							
July 9	5	116	72	9	18	9	224
25	5	54	98	27	0	27	206
Aug. 5	15	9	18	5	9	9	50
15	5	72	240	0	27	0	339
1928							
June 28	2	1,334	245	445	156	89	2,269
July 16	5	18	54	98	45	89	304
25	10	67	129	40	45	49	330
Aug. 7	5	89	116	45	45	63	358
Total	77						

TABLE XIX. *Seasonal variation of bottom fauna. Grapevine Point depression. Station 10* *21-25 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1923							
Aug. 18	6	52	15	0	0	0	67
1926							
July 14	10	1,049	14	80	5	0	1,148
24	10	191	14	360	27	14	606
29	10	343	23	190	40	23	619
Aug. 6	15	12	3	63	6	0	99
23	10	809	23	5	14	5	856
1927							
July 8	25	310	249	247	29	9	844
26	10	18	125	5	9	18	175
Aug. 12	5	267	27	18	9	0	321
1928							
June 28	5	2,302	214	525	80	0	3,121
July 24	5	18	134	89	116	36	393
Aug. 7	5	240	151	9	63	18	481
Total	116						

TABLE XX. *Seasonal variation of bottom fauna. Center of Third Sister Lake. Station 1* *18 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Oct. 20	5	5,866	27	0	151	0	6,044
26	5	7,529	0	0	196	0	7,725
Nov. 2	6	12,665	0	0	171	0	12,836
10	2	28,798	311	156	423	0	29,688
17	2	32,108	423	200	734	0	33,465
24	2	35,130	334	178	645	0	36,287
1927							
Jan. 5	5	41,730	36	9	80	0	41,855
Apr. 16	3	34,391	164	45	30	60	34,690
June 1	5	5,751	54	98	80	0	5,983
9	2	7,354	67	112	89	0	7,622
Aug. 27	5	1,520	116	63	196	0	1,895
Sept. 13	5	3,049	27	72	125	0	3,273
15	5	2,018	27	0	169	0	2,214
16	5	2,748	63	26	196	0	3,033
17	5	2,871	28	0	152	0	3,051
27	5	3,564	0	0	89	0	3,653
Oct. 4	5	3,476	0	0	285	0	3,761
11	5	3,333	0	0	311	0	3,644
18	5	6,915	0	0	320	0	7,235
Nov. 1	5	7,057	0	0	303	0	7,360
8	2	15,910	0	0	312	0	16,222
15	2	21,265	0	0	423	23	21,711
21	5	37,179	71	0	418	107	37,775
26	5	34,717	89	0	489	89	35,384
29	1	36,489	623	0	534	89	37,735
Dec. 6	1	47,818	1,023	0	312	89	49,242
1928							
Jan. 23	2	71,526	45	0	67	0	71,638
Feb. 22	1	68,705	0	0	45	0	68,750
Apr. 7	4	41,119	12	0	23	12	41,166
11	4	36,497	12	12	23	12	36,556
*25	25	9,251	294	13	*1,550	18	11,126
June 6	2	6,710	445	67	89	0	7,311
Oct. 10	5	2,694	0	0	151	0	2,845
Nov. 10	5	4,764	27	0	125	0	4,916
24	5	28,380	1,876	89	285	18	30,648
Dec. 15	5	57,372	2,399	70	223	89	60,153
1929							
Feb. 9	2	68,038	956	0	289	0	69,283
Total	163						

*Samples taken on this date from 16, 17, and 18 meters were combined.

TABLE XXI. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 2 *16-17 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 17	2	29,397	578	245	1,645	45	31,910
24	2	29,953	511	156	1,845	23	32,488
1927							
Sept. 27	5	1,120	18	0	1,813	0	2,950
Oct. 11	5	3,715	27	0	471	0	4,213
Nov. 8	2	13,444	0	0	689	0	14,133
15	2	21,576	23	0	578	45	22,222
22	4	23,542	256	0	978	89	24,865
26	4	26,042	400	12	1,145	90	27,690
1928							
Jan. 23	2	48,373	112	0	845	0	49,330
Feb. 22	1	53,595	90	0	623	0	54,308
Total	29						

TABLE XXII. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 3 *14-15 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1927							
Nov. 8	2	13,288	0	0	2,644	0	15,932
15	3	17,707	90	0	2,580	90	20,467
22	5	10,400	356	9	7,377	98	18,240
26	4	9,077	356	34	8,933	78	18,478
Dec. 6	2	11,158	911	45	9,400	0	21,514
1928							
Jan. 23	3	9,150	1,024	45	8,646	0	18,865
Feb. 22	1	9,866	845	45	9,200	0	19,956
Apr. 11	3	8,157	341	45	6,792	45	15,380
June 6	2	4,888	312	134	4,466	0	9,800
Oct. 10	2	11,266	0	0	267	0	11,533
Nov. 10	2	14,176	911	267	2,710	445	18,510
1929							
Feb. 9	2	37,996	711	23	245	24	39,000
Total	31						

TABLE XXIII. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 4 *11.5-13 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 2	2	15,620	200	67	9,644	45	25,576
10	2	3,045	2,090	645	6,690	200	12,670
1927							
Oct. 11	3	3,100	30	0	3,650	89	6,869
Nov. 8	2	13,910	67	23	13,310	23	27,333
15	2	3,555	533	0	11,666	134	15,888
22	3	2,314	1,780	149	12,770	134	17,147
26	3	2,536	1,424	89	9,180	208	13,437
1928							
Jan. 23	7	139	1,816	242	8,418	235	10,850
Feb. 22	10	116	1,960	147	11,137	120	13,480
Apr. 11	4	523	1,322	456	18,132	100	20,533
June 6	2	2,022	2,510	178	11,577	89	16,376
Oct. 10	4	4,400	0	0	3,233	0	7,633
Nov. 10	2	15,345	822	134	10,510	23	26,834
1929							
Feb. 9	2	1,245	3,644	0	16,020	67	20,976
Total	48						

TABLE XXIV. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 5 *9-10 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 10	2	2,600	4,355	867	5,133	578	13,533
1927							
Sept. 27	1	489	1,733	0	1,510	0	3,732
Oct. 11	3	757	920	0	149	282	2,108
Nov. 8	2	445	3,777	311	5,510	267	10,310
15	8	383	3,083	428	6,789	322	11,005
22	3	504	2,269	223	7,786	178	11,960
26	3	564	1,558	163	7,964	119	10,368
Dec. 6	1	667	933	134	7,066	311	9,111
1928							
Jan. 23	2	45	134	67	5,289	845	6,380
Feb. 22	1	89	178	89	5,422	1,022	6,800
Apr. 11	3	727	1,810	742	14,489	312	18,080
June 6	2	2,910	689	1,645	7,466	111	12,821
Oct. 10	2	2,933	178	0	3,844	0	6,955
Nov. 10	2	2,422	5,800	911	4,800	445	14,378
1929							
Feb. 9	2	0	1,800	134	8,022	311	10,267
Total	37						

TABLE XXV. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 6 *7-8 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 17	2	89	3,156	200	2,733	778	6,956
1927							
June 9	4	511	767	2,078	12,177	323	15,856
Sept. 27	5	4,204	6,142	507	1,254	1,369	13,476
Oct. 11	5	3,600	6,969	480	1,111	1,307	13,467
Oct. 18	2	1,555	4,400	845	1,378	200	8,378
25	5	311	4,595	791	2,044	676	8,417
Nov. 1	3	134	4,450	519	1,083	727	6,913
8	2	45	3,155	267	2,822	800	7,089
15	2	45	667	489	3,422	800	5,423
22	2	134	178	267	7,555	2,533	10,667
26	3	75	238	193	5,695	682	6,883
Dec. 6	2	0	156	311	4,577	712	5,756
1928							
Feb. 22	5	0	80	276	4,524	605	5,485
Apr. 11	3	225	2,848	2,195	5,962	1,757	12,987
June 6	2	445	734	2,755	16,598	289	20,821
Oct. 10	4	3,522	5,322	1,144	1,166	433	11,587
Nov. 10	4	234	1,056	645	1,900	4,010	7,845
1929							
Feb. 9	2	0	45	689	16,000	1,800	18,534
Total	57						

TABLE XXVI. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 7 *5-6 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 10	5	0	0	36	27	320	383
17	5	0	9	27	18	187	241
24	2	200	45	23	0	67	335
1927							
Sept. 27	10	631	151	23	143	462	1,410
Oct. 11	4	467	178	34	300	722	1,701
Nov. 8	2	67	156	445	400	1,244	2,312
15	1	134	223	667	45	978	2,047
1928							
Nov. 10	2	23	89	400	134	1,378	2,024
Total	31						

TABLE XXVII. *Seasonal distribution of bottom fauna. Third Sister Lake.*
Station 8 *3-4 meters*

Date	No. of Samples	Number per square meter					
		Corethra	Chironomus	Protenthes	Tubificidae	All Others	Totals
1926							
Nov. 17	2	0	45	112	0	145	802
24	1	0	45	134	0	267	446
1928							
Oct. 10	2	0	45	267	289	889	1,490
Nov. 10	2	0	0	67	200	178	445
Total	7						

TABLE XXVIII. *Depth distribution of bottom fauna. Third Sister Lake.*

Date	Depth meters	Number per square meter						
		Corethra	Chironomus	Protenthes	Tubificidae	Hydracarina	All Others	Totals
1926								
Nov. 10	5–6	0	0	36	27	80	240	383
	9–10	2,600	4,355	867	5,133	511	67	13,533
	11.5–13	3,045	2,090	645	6,690	0	200	12,670
	18	28,798	311	156	423	0	0	29,688
Nov. 17	3–4	0	45	112	0	0	645	802
	5–6	0	9	27	18	56	133	241
	7–8	89	3,156	200	2,733	400	378	6,956
	16–17	29,397	578	245	1,645	0	45	31,910
	18	32,108	423	200	734	0	0	33,456
Nov. 24	3–4	0	45	134	0	0	267	446
	5–6	200	45	23	0	0	67	335
	16–17	29,953	511	156	1,845	0	23	32,488
	18	35,130	334	178	645	0	0	36,287
1927								
Sept. 27	5–6	631	151	23	143	107	355	1,410
	7–8	4,204	6,142	507	1,254	649	720	13,476
	9–10	489	1,733	0	1,510	0	0	3,732
	16–17	1,120	18	0	1,813	0	0	2,950
	18	3,564	0	0	89	0	0	3,653
Oct. 11	5–6	467	178	34	300	12	710	1,701
	7–8	3,600	6,969	480	1,111	711	596	13,467
	9–10	757	920	0	149	0	282	2,108
	11.5–13	3,100	30	0	3,650	60	29	6,869
	16–17	3,715	27	0	471	0	0	4,213
	18	3,333	0	0	311	0	0	3,644
Nov. 8	5–6	67	156	445	400	200	1,044	2,312
	7–8	45	3,155	267	2,822	400	400	7,089
	9–10	445	3,777	311	5,510	267	0	10,310
	11.5–13	13,910	67	23	13,310	23	0	27,333
	14–15	13,288	0	0	2,644	0	0	15,932
	16–17	13,444	0	0	689	0	0	14,133
	18	15,910	0	0	312	0	0	16,222
Nov. 15	5–6	134	223	667	45	667	311	2,047
	7–8	45	667	489	3,422	578	222	5,423
	9–10	383	3,083	428	6,789	311	11	11,005
	11.5–13	3,555	533	0	11,666	134	0	15,888
	14–15	17,707	90	0	2,580	90	0	20,467
	16–17	21,576	23	0	578	45	0	22,222
	18	21,265	0	0	423	23	0	21,711
Nov. 26	7–8	75	238	193	5,695	0	682	6,883
	9–10	564	1,558	163	7,964	119	0	10,368
	11.5–13	2,536	1,424	89	9,180	208	0	13,437
	14–15	9,077	356	34	8,933	78	0	18,478
	16–17	26,042	400	12	1,145	90	0	27,690
	18	34,717	89	0	489	89	0	35,384
Dec. 6	7–8	0	156	311	4,577	0	712	5,756
	9–10	667	933	134	7,066	311	0	9,111
	14–15	11,158	911	45	9,400	0	0	21,514
	18	47,818	1,023	0	312	89	0	49,242

TABLE XXVIII. *(Continued)*

Date	Depth meters	Number per square meter						
		Corethra	Chironomus	Protenthes	Tubificidae	Hydracarina	All Others	Totals
1928								
Feb. 22	7–8	0	80	276	4,524	0	605	5,485
	9–10	89	178	89	5,422	400	622	6,800
	11.5–13	116	1,960	147	11,137	112	8	13,480
	14–15	9,866	845	45	9,200	0	0	19,956
	16–17	53,595	90	0	623	0	0	54,308
	18	68,705	0	0	45	0	0	68,750
Apr. 11	7–8	225	2,848	2,195	5,962	697	1,060	12,987
	9–10	727	1,810	742	14,489	225	87	18,080
	11.5–13	523	1,322	456	18,132	89	11	20,533
	14–15	8,157	341	45	6,792	45	0	15,380
	18	36,497	12	12	23	12	0	36,556
June 6	7–8	445	734	2,755	16,598	23	266	20,821
	9–10	2,910	689	1,645	7,466	111	0	12,821
	11.5–13	2,022	2,510	178	11,577	67	22	16,376
	14–15	4,888	312	134	4,466	0	0	9,800
	18	6,710	445	67	89	0	0	7,311
Oct. 10	3–4	0	45	267	289	134	755	1,490
	7–8	3,522	5,322	1,144	1,166	211	222	11,587
	9–10	2,933	178	0	3,844	0	0	6,955
	11.5–13	4,400	0	0	3,233	0	0	7,633
	14–15	11,266	0	0	267	0	0	11,533
	18	2,694	0	0	151	0	0	2,845
Nov. 10	3–4	0	0	67	200	89	89	445
	5–6	23	89	400	134	111	1,267	2,024
	7–8	234	1,056	645	1,900	345	3,665	7,845
	9–10	2,422	5,800	911	4,800	356	89	14,378
	11.5–13	15,345	822	134	10,510	0	23	26,834
	14–15	14,176	911	267	2,710	45	400	18,510
	18	4,764	27	0	125	0	0	4,916
1929								
Feb. 9	7–8	0	45	689	16,000	311	1,489	18,534
	9–10	0	1,800	134	8,022	245	66	10,267
	11.5–13	1,245	3,644	0	16,020	45	22	20,976
	14–15	37,996	711	23	245	24	0	39,000
	18	68,038	956	0	289	0	0	69,283

TABLE XXIX. *Depth distribution of bottom fauna. Douglas Lake.*

Locality	Date	Depth meters	No. Samples	Number per square meter							
				Corethra	Chironomus	Protenthes	Tubificidae	Sphaeriidae	Nematoda	All Others	Totals
	1926										
Grapevine Channel.....	June 25	13	5	0	9	9	0	0	0	0	18
		15	5	258	89	205	400	0	9	0	961
		16	5	231	116	347	27	0	0	0	721
	28	12	10	0	23	9	0	0	0	0	32
		13	10	5	40	18	0	0	58	0	121
		14	10	227	302	307	9	27	573	40	1,485
		15	5	98	125	205	45	0	178	0	651
South Fish-Tail Dep.....	July 7	14	1	134	0	89	45	0	0	0	268
		17	1	45	0	45	45	0	0	0	135
		18	1	90	45	0	0	0	0	0	135
		21	1	0	0	0	0	0	0	0	0
		23	15	131	27	21	60	0	0	3	242
	Aug. 10	15	5	0	71	18	0	0	0	0	89
		17	5	54	62	107	36	0	0	0	259
		18	10	36	9	45	40	0	0	0	130
		22	5	9	9	10	0	0	0	0	28
		23	10	5	9	0	5	0	0	0	19
	1928										
South Fish-Tail Dep.....	June 22	12	1	45	194	489	178	45	266	89	1,306
		18	2	800	67	1,000	89	0	0	45	2,001
North Fish-Tail Bay.....	July 2	4–8	20	0	0	0	0	0	0	0	0
		10	5	27	160	107	160	54	0	578	1,086
		10.5	5	116	18	53	169	36	0	418	810
		11	5	54	89	418	151	18	0	72	802
		12	2	356	90	289	45	45	23	0	848

TABLE XXIX. *(Continued)*
Depth distribution of bottom fauna. Douglas Lake.

Locality	Date	Depth meters	No. Samples	Number per square meter							
				Corethra	Chironomus	Protenthes	Tubificidae	Sphaeriidae	Nematoda	All Others	Totals
Roberts Point Dep.....	July 12	10	5	0	36	10	80	0	0	0	126
		15	5	382	651	229	0	134	0	125	1,521
		20	5	391	116	187	18	0	9	27	748
		21	5	222	89	34	98	0	0	0	443
		22	5	223	89	54	98	0	0	0	464
Fairy Island Dep.....	July 23	17	5	418	223	142	62	18	0	9	872
		20	5	142	151	10	240	0	0	0	543
		23	5	54	106	0	80	0	0	0	240
		28	5	18	45	0	18	0	0	0	81
Grapevine Channel.....	Aug. 4	10	5	0	0	0	0	0	0	0	0
		11	5	0	0	0	0	0	0	0	0
		15	5	0	0	0	0	0	0	0	0
		13	5	98	125	187	0	0	409	18	837
		14	4	56	167	522	45	0	189	11	990
		15	5	90	134	169	89	0	782	36	1,300
South Fish-Tail Bay.....	Aug. 4	10.5	5	0	36	9	18	0	0	36	99
		12	5	89	445	89	267	0	0	0	890
		13	5	45	445	356	329	27	0	54	1,256
		15	5	275	54	54	134	0	0	0	517
		18	5	160	62	45	152	9	0	0	428
		20	5	45	36	0	10	0	0	0	91
		23	5	27	18	0	9	0	0	0	54
Roberts Point Dep.....	Aug. 6	10	5	18	36	98	0	45	0	98	295
		11	5	143	45	27	36	0	0	63	314
		13	5	340	276	71	18	0	0	0	705
		20	5	187	258	36	178	0	0	0	659
		22	25	63	36	18	11	0	0	8	136

TABLE XXIX. *(Continued)*
Depth distribution of bottom fauna. Douglas Lake.

Locality	Date	Depth meters	No. Samples	Number per square meter							
				Corethra	Chironomus	Protenthes	Tubificidae	Sphaeriidae	Nematoda	All Others	Totals
Sedge Point Dep.	Aug. 9	10	5	0	0	0	0	0	0	0	0
		15	5	0	0	0	0	0	0	0	0
		18	5	107	27	0	45	0	0	0	179
		20	5	107	27	9	27	0	0	0	170
		23	5	72	18	0	17	0	0	1	107
		24	5	18	0	0	18	0	0	0	36
Roberts Point Dep.	Oct. 20	12	5	809	18	268	36	18	0	151	1,300
		14	5	1,555	107	320	27	9	27	223	2,268
		21	4	2,278	144	112	67	0	0	156	2,757
Grapevine Channel and South Fish-Tail Dep.	Oct. 20	10	5	0	9	10	0	0	0	0	19
		12	5	45	18	9	0	0	0	18	90
		13	2	889	90	45	0	23	223	867	2,137
		14	5	400	27	187	45	18	27	480	1,184
		15	5	3,875	169	391	187	0	18	551	5,191
		18	5	3,697	622	134	223	0	0	27	4,703
		22	5	3,448	80	36	18	0	0	0	3,582

TABLE XXX. *Occurrence of bottom fauna. Douglas Lake.*

Locality	Date	Depth meters	No. Samples	Number per square meter							
				Corethra	Chironomus	Protenthes	Tubificidae	Hydracarina	Sphaeriidae	All Others	Totals
	1923										
Maple Point Dep.	Aug. 18	16	15	0	0	0	0	0	0	0	0
	1926										
Grapevine Channel	June 21	17	2	422	245	334	0	0	67	112	1,180
	July 12	15	5	169	80	80	27	0	10	0	366
Roberts Point Dep.	Aug. 3	17	5	178	45	27	18	0	9	10	287
N. F. T. Bay, Sta. 70	20	11	10	63	231	36	230	10	5	27	602
N. F. T. Bay, Sta. 60	20	11	10	205	98	0	143	9	0	160	615
	1927										
Midway between Stations 30 and 50	Aug. 10	17	5	107	223	125	0	0	10	18	483

TABLE XXXI. *Summary of bottom samples. Green Lake, Kirkville, New York.*

June, 1925 *1-61 meters*

Date	Depth meters	No. Samples	Number per square meter											Remarks
			Cor.	Chir.	Prot.	Tub.	Hydra-carina	Suc.	Phy.	Val.	Gon.	Plan.	All Others	
June 17	21	5	0	0	0	0	0	0	0	0	0	0	0	Very black mud—H_2S
17	55–61	13	0	0	0	0	0	0	0	0	0	0	0	Much H_2S
18	5	5	0	0	0	0	0	0	0	27	0	240	0	Much gray marl
22	1	2	0	0	0	0	45	23	67	45	134	112	1,066	Much gray marl
26	3	2	0	0	0	0	112	0	0	0	23	178	134	
26	58	13	0	0	0	0	0	0	0	0	0	0	0	
27	4–8	15	0	0	0	0	0	0	0	0	0	0	0	Gray marl to black mud
27	10–30	15	0	0	0	0	0	0	0	0	0	0	0	Black mud—H_2S
27	40–61	35	0	0	0	0	0	0	0	0	0	0	0	H_2S very strong
28	60–30	25	0	0	0	0	0	0	0	0	0	0	0	H_2S very strong
28	20–5	15	0	0	0	0	0	0	0	0	0	0	0	
Total		145												

TABLE XXXII. *Bottom water analysis. Third Sister Lake, 1927.*

West End of Lake

Depth meters	Month: October 11					November 8					November 15				
	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.
0	15.8	7.7	5.58	2.0											
2	15.7	7.7	5.52	2.0											
4	15.5	7.6	5.50	2.1											
6	15.3	7.6	4.74	3.0	...	9.1	7.6	5.23	3.0	88	8.1	7.6	4.88	3.0	85
7.5	15.2	7.2	2.44	5.0											
8	14.1	...	....	...	...	9.1	7.6	5.09	3.0	87	8.0	7.6	4.53	3.0	85
10	....	...	....	...	...	9.1	7.6	4.88	3.0	89	8.0	7.6	4.53	3.0	86
12	....	...	....	...	...	9.1	7.6	4.88	3.0	89	8.0	7.4	4.32	5.0	86
14	....	...	....	...	...	...	...	....	...	..	7.7	7.4	4.04	5.0	89
16	....	...	....	...	...	...	...	....	...	..	7.6	7.1	2.09	10.0	95
17.5	....	...	....	...	...	...	...	....	...	..	7.4	7.0	0.07	17.0	101

Samples taken just above bottom, where water was respectively 0, 2, 4, 6, 7.5, 8, 10, 12, 14, 16, and 17.5 meters deep. All samples collected with Kemmerer sampler. Sampler lowered until it touched bottom, raised a few inches, and then closed. First water from sampler discarded in each case since it always was rather turbid.

TABLE XXXIII. *Vertical physical-chemical determinations. Third Sister Lake, 1927.*

Station 1 — *18 meters*

Depth meters	Month: October 11					November 8					November 15				
	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.	Temp. Cent.	pH	Oxygen cc. per l.	Free CO_2 ppm.	M.O. Alk. ppm.
0	15.8	7.7	5.58	2.0	80	9.0	7.6	5.55	3.0	82	9.2	7.6	5.44	3.0	84
1	15.7	...	....	...	..	9.0	...	....	...	..	8.8				
2	15.6	7.7	5.6	2.0	..	9.2	7.6	5.42	3.0	..	8.7	7.6	5.40	3.0	
3	15.6	...	....	...	..	9.2	...	....	...	..	8.7				
4	15.5	7.6	5.58	2.0	84	9.2	7.6	5.28	3.0	86	8.5	7.6	5.38	3.0	85
5	15.5	...	....	...	..	9.2	...	....	...	..	8.3				
6	15.5	7.6	5.16	3.0	..	9.2	7.6	5.22	3.0	..	8.2	7.6	5.02	3.0	
7	15.3	...	....	...	..	9.2	...	....	...	..	8.2				
8	14.4	7.4	4.60	4.5	89	9.2	7.6	5.20	3.0	86	8.15	7.6	4.75	3.0	85
9	11.2	7.3	....	...	..	9.2	...	....	...	..	8.15				
10	9.1	7.0	0.0	5.0	..	9.2	7.6	5.01	3.0	..	8.1	7.6	4.70	3.0	
11	8.2	...	....	...	..	9.2	...	....	...	..	8.1				
12	7.9	7.0	0.0	6.0	110	9.1	7.5	4.40	3.0	90	8.1	7.5	4.65	4.0	86
13	7.7	...	....	...	..	8.1	...	....	...	..	8.05				
14	7.6	7.0	0.0	7.0	..	7.5	7.3	0.61	5.0	..	8.0	7.5	4.40	4.0	
15	7.4	...	....	...	..	7.4	...	....	....	..	8.0	7.5			
16	7.4	7.0	0.0	10.0	..	7.4	7.1	0.14	10.0	..	7.9	7.1	2.23	8.0	
17	7.3	6.8	0.0	18.0	120	7.4	7.0	0.06	15.0	105	7.5	7.0	0.06	15.0	100
18	7.2	...	....	...	..	7.4	...	....	....	...	7.4				

TABLE XXXIV. *Physical chemical determinations. Douglas Lake.*

Date	Temperature Cent.			Thermocline		pH			Oxygen cc. per l.			Free CO_2 ppm.			M.O. Alkalinity ppm.		
	S.	u.l.H.	B.	Limits meters	Fall in Temp.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.	S.	u.l.H.	B.
1923																	
a. Aug. 18	20.7	N.S.	16.4	N.S.	N.S.	8.1	N.S.	7.3									
1926																	
b. June 25	16.7	N.S.	15.4	N.S.	N.S.	8.4	N.S.	8.2									
c. 28	17.3	N.S.	15.3	N.S.	N.S.	8.4	N.S.	8.2									
d. Aug. 20	20.0	N.S.	19.8	N.S.	N.S.	8.4	N.S.	8.0	6.0	N.S.	5.58	0.0	N.S.	0.0	125	N.S.	128
e. 20	20.0	N.S.	19.7	N.S.	N.S.	8.4	N.S.	8.0	6.0	N.S.	5.51	0.0	N.S.	0.5	125	N.S.	127
1928																	
f. July 5	22.6	N.S.	17.2	N.S.	N.S.	8.2	N.S.	7.6	5.65	N.S.	4.40	0.0	N.S.	5.0	...	N.S.	123
g. 5	22.6	N.S.	18.2	N.S.	N.S.	8.2	N.S.	7.8	5.58	N.S.	4.62	0.0	N.S.	3.5	...	N.S.	124
h. 5	22.6	N.S.	17.4	N.S.	N.S.	8.2	N.S.	7.7	5.65	N.S.	5.06	0.0	N.S.	3.5	...	N.S.	122
i. 7	23.3	18.3	17.3	5–7	4.1	8.2	8.2	8.2	6.02	5.50	5.0	0.0	0.0	0.0	...	134	126

a. Maple Point depression.
b. and c. Grapevine Channel.
d. North Fish-tail Bay, Station 70.
e. North Fish-tail Bay, Station 60.
f. North Fish-tail Bay, center of bay.
g. North Fish-tail Bay, near center of bay.
h. North Fish-tail Bay, midway between Hook and Diogenes Points.
i. North Fish-tail Bay, in cove behind Diogenes Point.

AN ECOLOGICAL STUDY OF THE TOBACCO BEETLE, *LASIODERMA SERRICORNE* FABR., WITH SPECIAL REFERENCE TO ITS LIFE HISTORY AND CONTROL

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CONTENTS

AN ECOLOGICAL STUDY OF THE TOBACCO BEETLE, *LASIODERMA SERRICORNE* FABR., WITH SPECIAL REFERENCE TO ITS LIFE HISTORY AND CONTROL

Introduction

The tobacco beetle, *Lasioderma serricorne* Fabricus (Order Coleptera, Family *Ptinidae*), is an insect of considerable economic importance. It belongs properly to that great group of insects which infest "stored food products." As the name suggests it is especially associated with tobacco, which it attacks only after it has been cured. Both the manufactured product and the unmanufactured leaf are infested. The place where the beetle does the greatest amount of damage is in the warehouse, while the tobacco is "aging." A badly infested hogshead may at times contain thousands of beetles. Cigar and tobacco factories, wholesale and retail establishments all suffer heavy losses because of this beetle. As a rule it can be found at any season in one stage or another in all such places. In general, infestation is heavier in the warmer climates and in the better grades of tobacco.

Damage caused by the beetle to tobacco is due: (1) to the actual amount of tobacco consumed by the larvae; (2) to production of refuse material, dead bodies, dust, and other products which cause tobacco to be of less value or render it worthless; (3) to holes cut in high grade tobacco, such as wrappers, which greatly affect its value, and (4) to natural prejudices among consumers to insects in tobacco.

Tobacco is not the sole food for the tobacco beetle. Back and Cotton (1927) state that the insect feeds upon many kinds of dried vegetable substances—such as ginger root, coriander seed, ground peppers, beans, wheat, flour or breakfast foods left undisturbed in storage for long periods, dried plants, and furniture materials (such as Spanish moss, palm fiber, flax tow, kapok, and sea moss). It has been reported as infesting cayenne pepper, hides, shoe leather, talcum powder, silk upholstering, spices, and even pyrethrum powder. Baker's yeast is a favorite food of the beetle.

Several economic entomologists have studied the tobacco beetle, but their work has been directed toward control. The life history and behavior of the beetle have either been ignored or dealt with briefly. When an insect is to be controlled, a thorough knowledge of its life history is fundamental and this involves a study of behavior. The tobacco beetle is well adapted to laboratory experimentation. It can be cultured successfully on a single type of food; it is easily controlled; its life cycle is short; it is small and can be easily obtained.

The object of the work described in the present paper was to study the tobacco beetle from an ecological point of view in order to obtain information that would prove of scientific interest and also be of practical value. Experiments which relate to life history, behavior, and control are described. The temperature, humidity, and food were controlled. Each of these factors has an optimum condition. Variations either above or below this tend to lengthen the life cycle. The optimum conditions were determined and the limits of toleration for the factors studied were investigated, especially with reference to temperature and humidity.

Behavior was investigated both in the laboratory and under natural conditions. The influence of factors such as different types and grades of tobacco, absence of particular materials, physical forms of substances, and odors were investigated.

Certain experiments were carried out in an endeavor to arrive at the most efficient method of destroying the tobacco beetle, or at least hold it in check. In this connection natural enemies, high and low temperatures, and fumigants were investigated. An effort was made to evaluate fumigants now in general use.

The writer's experiments extended over a period of two and one-half years, commencing January 1, 1928. They were carried on at Duke University, under the direction of Dr. A. S. Pearse and Dr. F. G. Hall, to whom the writer is deeply indebted for many suggestions and criticisms. Dr. P. M. Gross made many helpful suggestions; Mr. Alfred Hall gave friendly council, especially in relation to fumigants and analysis of tobacco samples; Mr. L. F. Dixon assisted in the grading of tobacco and Dr. E. P. Jones helped in the construction of apparatus and in calculations in connection with fumigation experiments.

HISTORICAL

The tobacco beetle was described from North America in 1792 by Fabricus. The following synonymy is given by Jones (1913):

Lasioderma serricorne Fabr. Ent. Syst. (1792), 1. 241; muls., Ann. Soc. Linn. Lyon (1864), 12, 1, Pl. 1, fig. 10. Lec., Proc. Acad. Nat. Sci. Phil. (1865), 238.
Lasioderma flavescens Dahlb., Dej. Cat. 3. ed. (1837), 129.
Lasioderma rufescens Sturm, Cat. (1826), 206.
Lasioderma testaceum Duftschm., Fauna Austr. (1859), 3. 46; Sturm, Deutschl. Fauna (1837), 11, 89, pt. 237, fig. P. Q.

Chittenden (1896) reared a generation of beetles in 47 days. Metcalf (1909) mentioned the insect as being a pest in tobacco in North Carolina, but did not report any experimental work on its life history. The first work of ecological importance was reported by Jones (1913) who worked in the Philippines. He found that the length of the egg stage varied from 4 to 10

days, the average length of the larval stage, 50 days, and the average length of the pupal stage 12.5 days. Counting 6 days as being the average length of the egg stage, this gives an average of 68.5 days for the length of the life cycle. Runner (1917) studied variations in the length of the life cycle. He reported that the time required to complete the life cycle depends mainly upon temperature. It may be as short as 45 days and normally varies in summer from 45 to 70 days. His account shows that the eggs hatch in from 6 to 10 days, the larval period extends through 30 to 50 days, and the pupal period from 6 to 10 days. He states further that the beetle thrives best where the temperature and humidity are high and in tobacco or other food substances protected from rapid evaporation. Back and Cotton (1927) state that the life cycle ranges from 42 to 70 days, but do not give the type of food they used or the temperature or humidities during their observations.

None of these reports give any data on the actual length of the life cycle, or any of its stages, under controlled conditions. The humidity and temperature were apparently that of the room in which the experiments were carried on and various types of tobacco served as food.

Temperature was discussed by Blackmann (1905) as a factor in the growth of insects. He states that for every rise of 10°C. the rate of metabolism is approximately doubled or trebled; that at high temperature (30°C. and above) the initial rate of metabolism cannot be maintained but decreases regularly; that the higher the temperature, the more rapid is the rate of decrease; that the decrease at any given temperature is at first rapid and subsequently becomes slower. Bachmetjew (1907) assumed that a certain concentration of body fluids was associated with the most rapid metabolism. Sanderson (1910) recognized humidity as a factor in the growth of insects. Headlee (1914) reported that a low per cent of moisture retarded metabolism, and that a high per cent increased it. Chapman (1920) states that *Tribolium confusum* Duval has its egg stage shortened from ten to five days by a rise of from 24° to 34° C. and that this beetle will develop one generation after another throughout the year. He also discovered that the life cycle may be prolonged by a reduction of the amount of moisture and also by a limitation of the quantity or quality of the food. Thus the length of life and the number of broods may be altered by changing any one or all of these three factors.

The codling moth has been extensively studied. Isely and Ackerman (1924) report that the average duration of the life of this insect in the different stages varies throughout the season with variations in temperature. Shelford (1927) reports the following factors as affecting the rate of develment of this insect: (1) variability of temperature and humidity; (2) rainfall which soaks the larvae or pupae; (3) wind or air movement; (4) quality and intensity of light; (5) food; (6) mechanical stimuli; (7) seasonal march of temperature and humidity.

To control the tobacco beetle, several different gases and other substances have been used. In 1909 Metcalf recommended the use of carbon bisulphide, gasoline, and hydrocyanic acid gas. Jones (1913) published an excellent account of control measures for the tobacco beetle. He recommended the use of carbon bisulphide, hydrocyanic acid gas, and high and low temperatures. He says that the cigarette beetle in all its stages can readily be killed by carbon bisulphide of the concentration of 14.4 grams per cubic meter in air tight vessels, where the fumes of carbon bisulphide come in direct contact with the insect. When beetles are protected in a mass of tobacco, a greater quantity of carbon bisulphide must be used. Experiments proved that 32 grams of carbon bisulphide per cubic meter were quite effective, but in practical work slightly more (40 grams) were recommended. In regard to the hydrocyanic acid gas Jones stated that 30 grams of potassium cyanide per cubic meter was sufficient, but he recommended the use of one kilogram of the cyanide to each 32.4 cubic meters if complete fumigation was desired. Runner (1917) has emphasized cold storage, freezing during winter, heat, steam, trapping and fumigation as means of control. He prefers hydrocyanic acid gas for fumigation. The dosage called for in his formula is 10 ounces of potassium cyanide or sodium cyanide per 1000 cu. ft. He also recommends carbon bisulphide; not less than 64 ounces of the liquid to each 1000 cu. ft., and used Roentgen Rays in the control of the pest. In more recent years new gases have been experimented with. Cotton and Roark (1928) made a favorable report on ethylene oxide for insects infesting stored food products. The same authors (1927) reported ethylene dichloride-carbon tetrachloride mixture as being efficient. Strand (1926) recommended chlorpicrin as being effective against beetles.

Tobacco beetles that infest stored foods are usually buried in them. A good many may be observed on the outside or flying about, but the majority of eggs, larvae, and pupae will be hidden from view. A fumigant that is effective must kill not only the adults at large, but also the stages buried in the food. In short, it must possess penetrative powers. Apparently no investigators have experimented on the fumigation of tobacco hogsheads. This field should be studied further. The gases recommended as fumigants should be tested with tobacco and under ordinary warehouse conditions.

EXPERIMENTAL METHODS

The experiments reported in this paper were controlled as far as possible. Such factors as temperature and humidity were kept constant.

LIFE HISTORY SERIES

In the study of life history it was desired to ascertain the effects of various degrees of humidity at the same temperature. It was desirable that a whole series of tests be carried on at the same time. An apparatus was de-

signed to do this (Figs. 1, 2). It consisted of an insulated box that could be kept at a constant temperature and a series of jars in which different humidities were maintained.

This box was made of beaver board, with sufficient wood braces to give stability. A false end separated the heating units from the chief compartment. In this was a circular opening large enough to accommodate an electric fan, which forced the air out of the experimental chamber and circulated it above as the arrows indicate. The heating unit consisted of four 75-watt electric light bulbs controlled by a thermostat which proved quite dependable. When accurately regulated, there was a normal variation of about 1°F. The box had a full size sliding door in front, which enabled its contents to be ex-

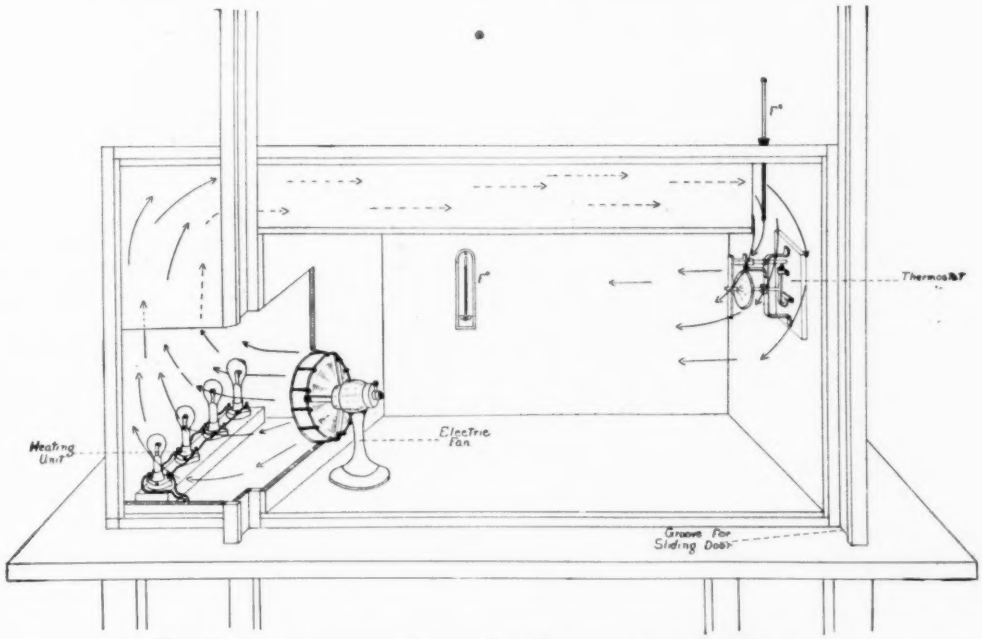

FIG. 1. An apparatus for maintaining constant temperature.

amined with ease. The fan was allowed to run during the entire time of the experiment. This caused the air on the inside of the box to be continuously circulated and resulted in uniform temperature in all parts of the box.

The apparatus for humidity control is shown in Fig. 2. It consisted of two gas washing bottles of 500 c. c. capacity. A and A' connected with two experimental chambers I and I'. The reaction chamber I' consisted of a 16 ounce screw cap jar. The connections were glass rods sealed into the lids with sealing wax. Experimental chamber I was identical with I' except that it had a capacity of 8 ounces. The whole was connected in series. The last chamber (1) was connected to a glass manifold (9) which in turn was joined to a Vacuum exhaust tube. This unit was complete for a single per cent of humidity. As many units were used as there were different percentages of

humidity desired, all of the units being connected to the same glass manifold (9).

To obtain the desired humidity, the air was conditioned by Wilson's (1921) method. This has been used by Coleman and Fellows (1925) to ascertain the moisture content of cereal grains and flaxseed in atmospheres of different but constant humidity. The desired humidity was obtained by using the very best grade of sulphuric acid and bringing it to the proper density, as shown in the tables and graphs in Wilson's paper. The acid was then poured into both bottles A and A' to the same level. Glass wool was used to filter the air drawn through the acid solution. An important precaution es-

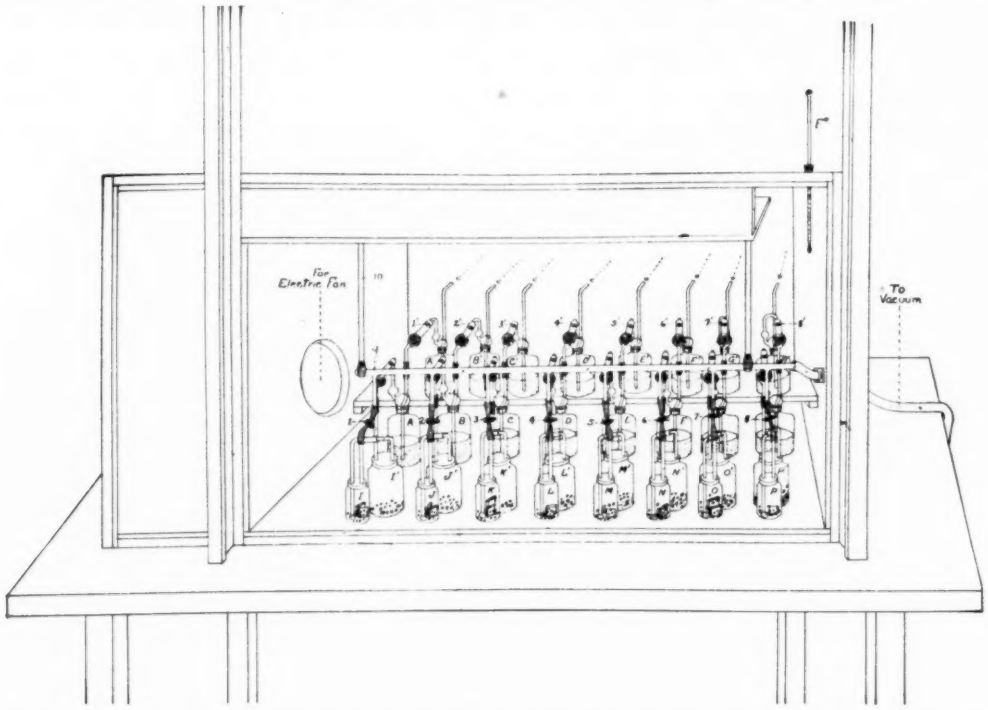

FIG. 2. Apparatus used in determining life cycle under various conditions. *A, A'-H, H',* gas washing bottles containing sulphuric acid solutions giving various percentages of humidity; *I, I'-P, P',* jars in which the bettles were placed; *1-8,* set screws for regulating the vacuum; *1-8',* calcium chloride tubes; *9,* glass manifold; *10,* support for manifold.

sential for the successful operation of this apparatus was the introduction of a calcium chloride tube (I') between the acid bottle (A) and the experimental jar (I'). This tube was plugged with cotton and granulated zinc. The cotton plug was placed in the end of the tube nearest the acid, and was for the purpose of taking up any acid that might be brought over into the experimental chambers. The granulated zinc was for the purpose of interacting with any acid that escaped the cotton.

In this series of experiments eight solutions were prepared to give humidities of 00, 15, 30, 45, 60, 75, 90, and 100 per cent. Such a series of bottles

was placed in each of three temperature boxes as shown in Fig. 2. The bottles producing various degrees of humidity were arranged in the following order, A (00%), B (15%), C (30%), D (45%), E (60%), F (75%), G (90%), H (100%). When assembled as shown in the plate, experimental chambers, I and I' were exposed to humidity of 00%, J and J' to 15%, K and K' to 30%, L and L' to 45%, M and M' to 60%, N and N' to 75%, O and O' to 90%, P and P' to 100%. The series of bottles for each humidity was separate and took its air directly from the box. Set screws (1-8) controlled the flow of air through each jar. During the entire period of the experiment the temperature of each box was regulated, and conditioned air was continuously drawn through each experimental chamber.

GENERAL PLAN OF LIFE HISTORY EXPERIMENTS

In the life history experiments it was desired to discover two things: (1) the actual length of the life cycle from adult beetles to adult beetles; (2) and the actual percentage of beetles that would become adults from a known number of eggs under the same conditions when food, humidity and temperature were constant. To obtain facts concerning the former an experiment was started with 30 adult beetles in each container. Only active beetles were chosen. The 16 ounce jars used contained 10 grams of tobacco as food. The 8 ounce jars contained 5 cakes of magic yeast. The beetles were introduced into the jars, which were then sealed. Each unit was tested for air leaks. The experiment was carefully watched from day to day. The first time that a new adult beetle was observed in any jar was taken as the actual length of the life cycle for the beetle at that particular degree of humidity. To discover the number of adults which developed from counted lots of eggs, an experiment was started with one hundred eggs, all laid within the previous 12 hour period. It was found that by catching several hundred beetles and placing them together in a large jar overnight along with several leaves of tobacco, great numbers of eggs could be obtained. The jars were then sealed and the experiment carefully watched. When the beetles began to emerge as adults, they were counted until the total number had been obtained. This gave the per cent based on one hundred eggs for each humidity.

ODOR SERIES

The apparatus (Fig. 3) used for experiments on odors was a modification of the type used and described by McIndoo (1927), and called by him, "An Insect Olfactometer." It consists of a specially prepared glass "Y" tube (5) connected directly to the exhaust through tube (6). Vacuum was obtained by a water-pump. A stand (3) supported a small glass container (4) which held the beetles. Beetles were induced to move from 4 toward (A) and (A') by a green light. Six gas washing bottles (A, A', B, B', C, C') of 250 c. c. capacity were connected as shown in the figure. The first pair (A) and (A')

were for the purpose of catching beetles, after they had reacted to an odor and passed through the tubes (5). The second pair (B) and (B') were the bottles in which the odorous substances were placed. The third pair (C) and (C') were bottles filled with distilled water. The odors of tobacco were derived from hot water extracts and also from dried leaves. When the hot water extracts were used the liquid carrying the odor was placed in one bottle and an equal amount of distilled water in the bottle on the opposite side. This was done in order that the flowing air would meet with the same resistance on both sides. In testing the odors of tobacco leaves one bottle contained the leaves and the other one clean air. Preliminary experiments showed that an equal amount of air would not be drawn through the two arms unless the apertures were regulated. The vacuum was regulated first at the water pump. Bottles (C) and (C') were filled to the same level with distilled water, and set screws (1) and (1') were placed on the rubber tube connecting these bottles with the rest of the apparatus. By regulating the screws the same amount of air could be drawn through one side as the other. This is an important feature in connection with the apparatus, because if more air was drawn through one arm than the other, such inequality might influence the reactions of the beetles. McIndoo states that, "The principle involved is to attract the insects equally towards the entrances of the forks by the light stimulus, but when they are ready to enter these forks they are influenced unequally by the odors drawn through the forks. One fork serving as an attractive or repellent side and the other fork as the control side. The whole apparatus is so constructed and manipulated that the interfering factors are practically controlled, leaving only the olfactory responses to be recorded."

GENERAL PLAN OF ODOR EXPERIMENTS

All odor experiments were run in a dark room, the only light present being the green one to attract the insect down the runway. The insects used in these experiments were in the adult stage. Care was exercised to use only those healthy and active. Preliminary experiments showed that inactive beetles often would not leave the reaction chamber. The beetles were not enclosed in the small vials before being used, as this tended to render them less active. An odor experiment, as such a term is used in this paper, consisted of the reactions of approximately 120 beetles. Hot water extracts were aerated before they were tested, and all tobacco in the leaf form was conditioned by drawing air saturated with water through it for a period of 24 hours before placing it in the odor chamber.

Four groups of experiments were carried out. This reduced experimental error. Thirty beetles were tested together and these were allowed thirty minutes in which to react. At the end of that time all of the beetles remaining in the reaction chamber were regarded as not reacting. During

the experiment the tube containing the odor tested was changed from side to side. If it were on the left side while the first group of beetles was being tested, it was placed on the right during the tests on the second, thus, any influence due to the position of the apparatus was compensated for. When the bottle containing the odor was changed, the "Y" tube was thoroughly aerated by drawing air through it to remove any traces of odors. Any single beetle was tested only once. At the conclusion of the experiment the results obtained from the four groups were consolidated and reported as a single experiment.

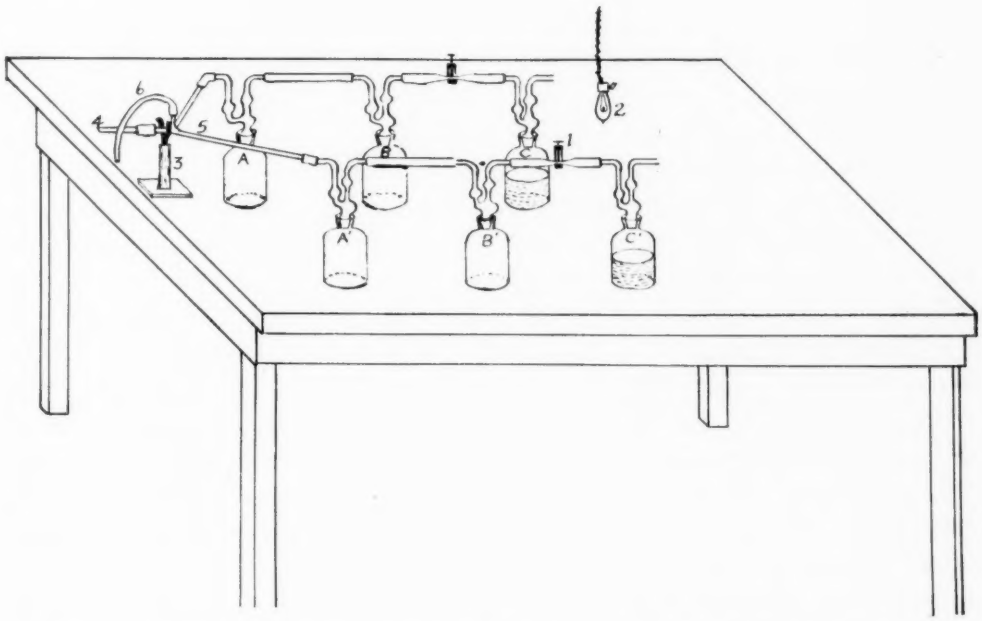

FIG. 3. Insect Olfactometer (after McIndoo). *A, A'; B, B'; C, C'*, gas washing bottles; *1*, set screw; *2*, electric light; *3*, support; *4*, tube (for containing insects before they responded); *5*, glass "Y" tube; *6*, connection with vacuum.

CULTURE METHODS

Several methods were employed in an effort to culture tobacco beetles. Tobacco and yeast were tried as the food and the former was not nearly so satisfactory as the latter as a culture medium. It is quite probable that the compactness of a magic yeast cake is a factor which renders it desirable. When raised in small jars in the laboratory tobacco was found to be a satisfactory food only when it was used in great quantities. Yeast was the substance in which the beetles were reared during routine experiments. This food material has been used for some time for insects. Richardson (1926) mentions it in connection with the Mediterranean flour moth. Mr. F. S. Chamberlin used yeast to culture the tobacco beetle in his laboratory at Quincy, Florida, during 1928. The method employed by the writer was to put about

thirty yeast cakes in a half-gallon Mason fruit jar, and innoculate it with from 30-50 adult beetles. The beetles were held in the jar by placing organdie over the top or lightly screwing on the lid without a jar rubber. The jars were kept at a temperature of from 28°-35°C. Humidity was an important factor in rearing beetles. If it was too high, mold which killed the beetles grew and if the stock jars were placed in too dry an atmosphere the beetles would not mature. It was found that the ordinary atmosphere of an oven which maintained a temperature of about 33°C. was suitable for rearing beetles.

FUMIGATION SERIES

To carry out the experiments on fumigation a special piece of apparatus was designed (Fig. 4). The metal container shown was made of galvanized iron. It was three feet long with a total capacity of 8.58 cu. ft. It was built with a close fitting top that rested in a paraffin trough. Melted paraffin was poured into the trough and allowed to harden, thus producing an air tight compartment. Through the center of the top there projected down into the container a stirrer made by attaching a six inch fan blade to the end of a metal rod. The motive force of the stirrer was obtained from air pressure directed against a wind mill attached to the upper end of the rod. The whole stirring apparatus was sealed by an oil seal through the top, thus preventing the escape of gases. The function of the stirring apparatus was to distribute the gases uniformly after the fumigant had been administered. This was accomplished by running the fan for fifteen minutes at the beginning of each experiment. The gases used for experiments were all generated and run into the compartment from the outside. There are two outlets for this purpose, one in the top and one in the bottom. When a gas heavier than air was used, it was administered through the bottom outlet. The top outlet was opened during the actual ingress of the gas so that a volume of air equal to that of the gas would be displaced. This was done to prevent increase of atmospheric pressure, and consequently obtaining results not comparable to actual conditions in a warehouse undergoing fumigation. The reverse process was followed if the gas was lighter than air. In every case both inlet and outlet were closed by means of clamps after a gas had been administered. The air was then thoroughly mixed by the fan.

The purpose in these experiments was to simulate the conditions in a warehouse as nearly as possible, and hence it was decided to test all beetles in tobacco. Two presses were constructed by using sheet metal pieces thirteen inches square and one-eighth of an inch thick. Two holes were bored through each piece of metal equidistant apart and through these holes long iron-bolts with screw nuts were inserted. Such a press when assembled consisted of top and bottom piece of metal with tobacco between. Each was a cube which measured thirteen inches on each edge. The same amount of

tobacco was always used (23.6 lbs.). This was compressed to a depth of thirteen inches, by tightening up the screws, and the resulting pressure was the same as that on the tobacco in an ordinary hogshead.

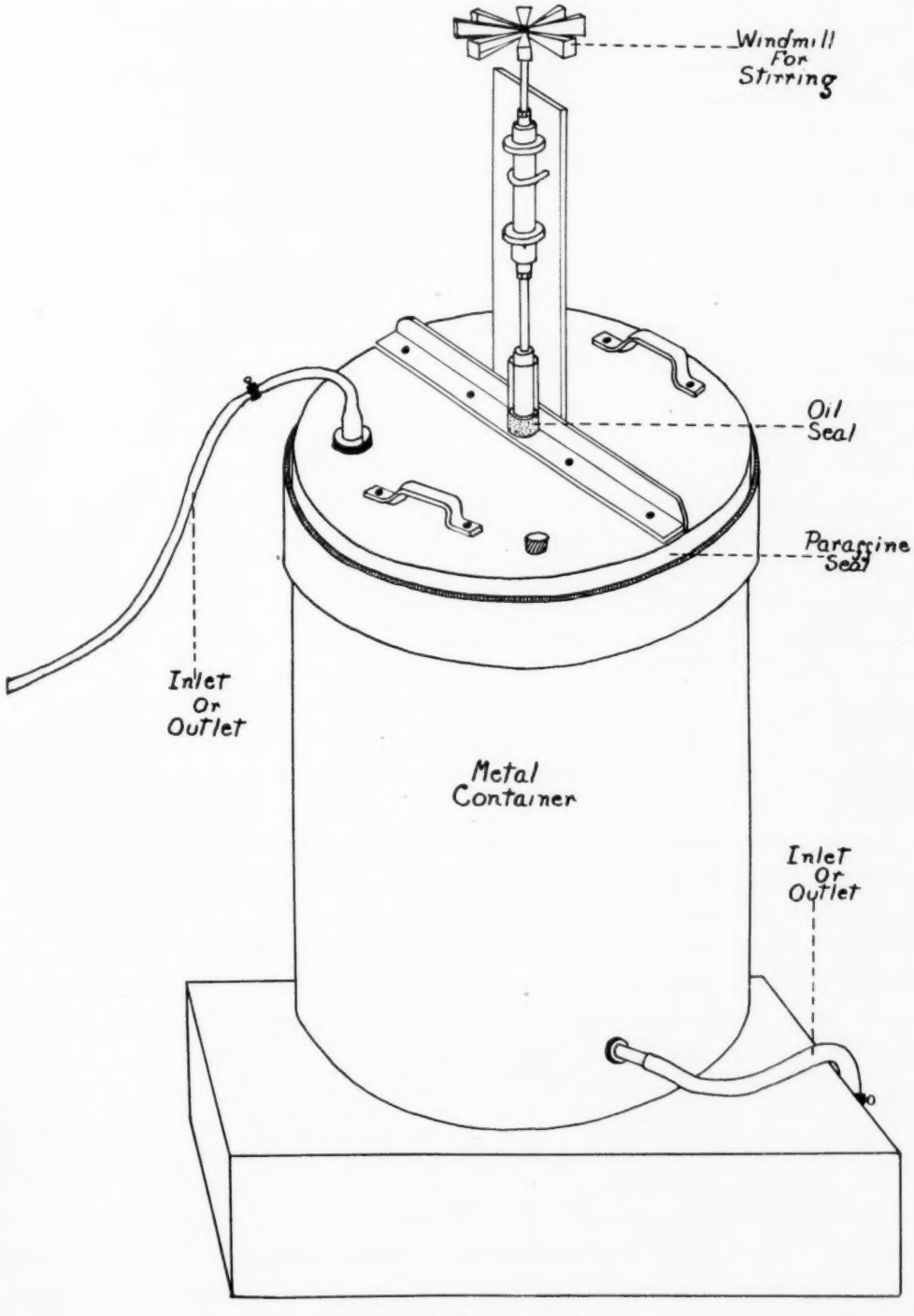

FIG. 4. Fumigation tank.

PLAN OF FUMIGATION EXPERIMENTS

All stages of tobacco beetles were used at various times in the fumigation experiments, but the larvae were the usual subjects for tests. Each larva was placed in a Size 0 gelatine capsule. A small amount of moist tobacco was inclosed with each insect to serve as food, for the larva attempted to escape unless such material was present. Five small needle holes were punched in either end of the capsule and five others around the equator. This assured the passage of the gas into the capsule. The capsules were placed in holes in wooden sticks. The sticks measured one by one by thirteen inches. Thirteen holes were bored through each stick. These were one inch apart commencing one-half inch from the end. The capsules fitted these closely. Three sticks were placed in a press. The first one was placed one-fourth of the distance from the bottom; the second in the middle and the third, three-fourths of the distance. By this means it was possible to determine the distance the gas penetrated. Each press, therefore, held a total of thirty-nine capsules containing larvae, which made a total of seventy-eight capsules and larvae in the container for each fumigation test. Six or more larvae in capsules were also placed in open pasteboard boxes within the experimental tank to serve as controls. Adults, eggs, and pupae were also tested in these control boxes.

In preparation for the fumigations the larvae were caught and placed in capsules a short while before they were actually put in the tobacco press. The temperature was recorded. The atmospheric humidity was also determined with a sling psychrometer at the beginning and end of each experiment. Fresh leaf tobacco was used loose in each experiment, not tied up in bundles or hands. It was thought best to use it in the loose leaf form because it would pack closer in the press and consequently the results would be more uniform.

All of the experiments extended through a period of 24 hours. The sticks were removed from the tobacco presses when each test was over and beetles were examined. A record of the condition of each larva was made. After examination the beetles were returned to the capsules, which were once more inserted into the sticks, removed from the container and were laid aside for one week before reëxamination. Many larvae would recover during the week. Those that had been actually killed were shriveled up and of a dark color at the second examination.

EXPERIMENTAL RESULTS

Life History Series

Normal Length of Tobacco Beetle Life Cycle

A preliminary experiment was carried out to determine the normal length of the beetle life cycle. The temperature and humidity were not controlled

in any way. An effort was made to duplicate as nearly as possible the natural conditions under which the beetle lives. The experiment was commenced on June 21, 1928 under the ordinary conditions in a laboratory room. Tobacco and yeast were used as food. Four glass jars of one pint capacity served as containers, two for the tobacco and two for the yeast. Ten grams of tobacco, Quality D. E. N. C., was placed in two of the bottles and ten grams of magic yeast in the other two. Ten pairs of beetles were introduced with the food into each jar. Strips of organdie held over the top of the jars by rubber bands prevented the escape of the beetles, yet allowed the entrance of air.

The result of this experiment was that in one jar of tobacco a new generation of beetles came to maturity in 57 days, and in the other in 65 days. This gives a variation of 8 days between the maturity of beetles in the same food substance and under essentially identical conditions. The time required for the beetles to reach maturity in the yeast was much shorter: in one jar, 40 days and in the other, 41. The average time required for the completion of the life cycle in yeast was 20.5 days less than the average time required in tobacco.

TABLE I. *The life cycle of the tobacco beetle in days under controlled conditions.*

Temperature °C.	Percentage of Humidity							
	00	15	30	45	60	75	90	100
24	00*	00*	00*	63	59	55	62	00**
28	00*	00*	00*	46	41	36	42	00**
32	00*	00*	00*	36	31	28	32	00**
36	00*	00*	00*	38	34	29	00**	00**
40	00*	00*	00*	00*	00*	00*	00*	00*

*Did not come to maturity.
**Material molded.

This experiment shows, that at room temperature, and under similar conditions the life cycle of the tobacco beetle is much shorter in yeast than in tobacco.

DETERMINATION OF THE LIFE CYCLE UNDER CONTROLLED CONDITIONS

The total length of the life cycle of the tobacco beetle was studied, with magic yeast as the food, at 5 different temperatures and in various humidities: 24°C. (75°F.), 28°C. (82°F.), 32°C. (90°F.), 36°C. (97°F.), 40°C. (105°F.). The life cycle was not completed in the following humidities: 00, 15, 30, and 100 per cent. This was true at all 5 temperatures.

Table I gives the results obtained at the 5 temperatures and in 8 humidities at each temperature, when magic yeast was the food used. From this table it is apparent that 40°C. was fatal to the beetle. Fig. 5 illustrates the effect of humidity on the length of the life cycle at different temperatures.

It is plain that 45, 60, 75, and 90 per cent humidity had about the same relative effect at each temperature. The optimum humidity for the beetle at every temperature was 75 per cent. The other three percentages of humidity at which beetles completed their life cycles ranked in the following order: 60, 90, and 45 per cent. Fig. 6 shows the effect of temperature on the length of the life cycle at different degrees of humidity. The optimum temperature was 32°C. There is a gradual increase from 24°C. to the optimum at 32°C., a slight falling off to 36°C. and then a rapid decline and no completion of life cycles at 40°C.

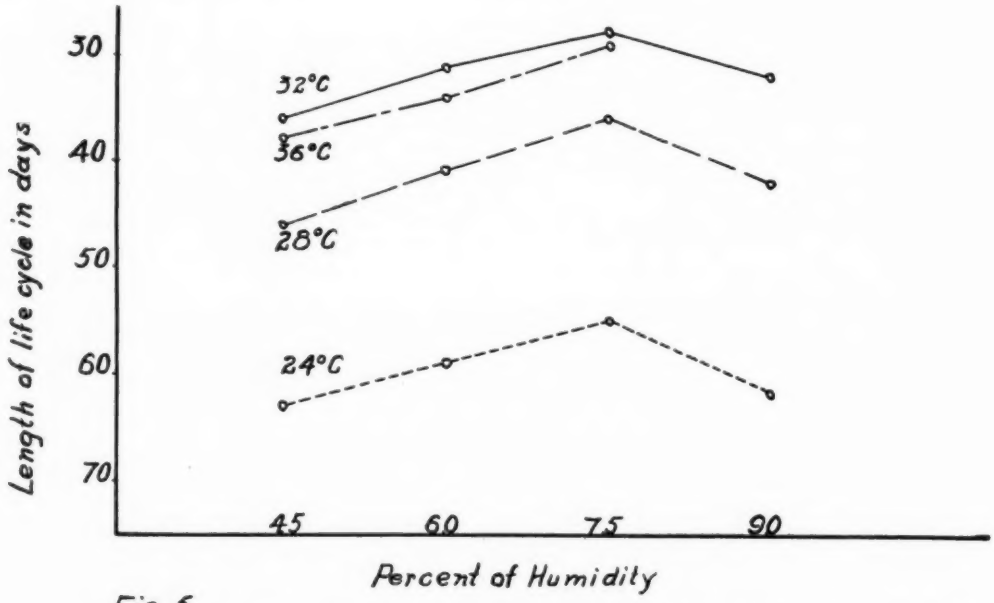

Fig. 5
Showing the effect of temperature and humidity on the life cycle of the tobacco beetle

All of the results discussed were obtained when magic yeast was used as the food. Tobacco was also tried with yeast as a control. Owing to the fact that tobacco molded in a number of instances the figures are not as complete as in the case of the yeast. At 32°C. the length of the life cycle was found to be as follows: 46 days in 75 per cent humidity, 49 days in 60, and 55 days in 45. When tobacco was used as food and the humidity was 75 per cent the life cycle was 18 days longer than when yeast was used as food. In 60 per cent humidity the cycle was 18 days longer; and in 45 per cent, 19 days longer. The same effect from various degrees of humidity were obtained in tobacco as well as in yeast, the chief difference being a lengthening of the life cycle by 18 days when tobacco was the food used. The life cycle in 75 per cent humidity is 70 days long at 24°C., 55 days at 28°C., and 49 days at 36°C. Comparing these figures with those in Table I, there is found a uniform difference of from 18 to 20 days more in tobacco than in yeast.

The results of these experiments under controlled conditions show: (1) that low humidities (00, 15, and 30 per cent) will not permit beetle growth; (2) high humidities (100 per cent) are unfavorable; (3) a constant temperature of 40°C. is fatal to beetles in every stage; (4) the optimum temperature is 32°C.; (5) at temperatures below and above the optimum the life cycle is longer; (6) there is a much shorter range above the optimum than below that will permit beetle life; (7) humidity exerts a decided effect on the length of the life cycle; (8) 75 per cent humidity is optimum; (9) humidities below and above the optimum lengthens the life cycle and (10) similar results were obtained when tobacco was used as food, the chief difference being that the life cycle is longer than when yeast is used.

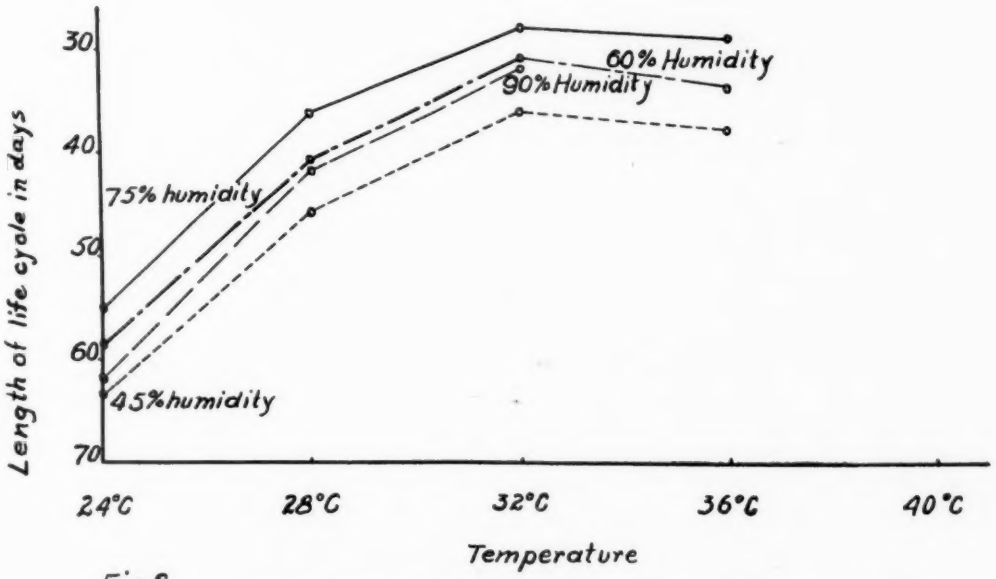

Fig. 6
Showing the effect of temperature and humidity on the life cycle of the tobacco beetle

DETERMINATION OF THE LENGTH OF THE SEPARATE STAGES IN THE LIFE CYCLE UNDER CONTROLLED CONDITIONS

The length of the separate stages in the life cycle of the beetle were studied at 32°C. and in 45, 60, 75, and 90 per cent humidities. Magic yeast was used as food. All stages were kept separate. The eggs were obtained within a twelve hour period of starting the experiment. The larvae were taken from those that emerged on a single day. The pupae used were obtained from larvae pupating within a twelve hour period. Adults were captured immediately upon emergence. Six beetles in each stage were used for the experiment. The experiment was run in desiccator chambers with sulphuric acid diluted so as to obtain the desired humidity. All stages were put in low glass dishes covered with organdie before being placed in the desiccators.

The experiment was run in a box regulated to maintain a temperature of 32°C. It is quite probable that daily handling, which was necessary in this case, caused the total time required to complete the life cycle to be lengthened.

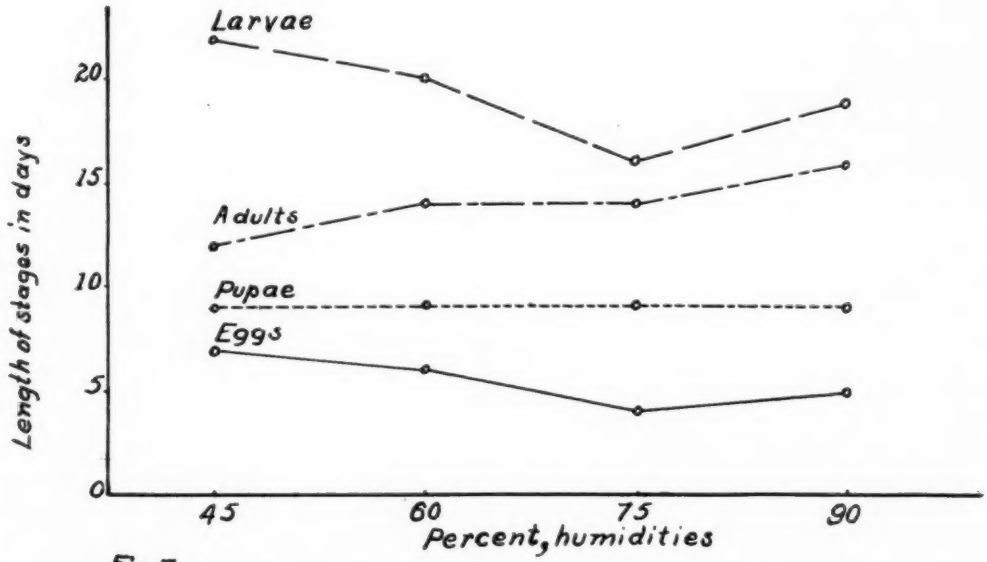

Fig. 7
Showing the length of the separate stages in the life cycle at 32° C and different humidities

Results are shown in Table II and Fig. 7. From these the following facts are apparent: (1) that 75 per cent of humidity is nearer the optimum than any others tried for the eggs and larvae; (2) the length of the incubation period of the egg is lengthened in low humidities; (3) the life of the larva is longer in low humidities; (4) the pupa is affected very little by humidity; and (5) the length of the life cycle of the tobacco beetle as determined by a particular humidity is due largely to the effects on the egg and larva.

TABLE II. *The length* of the separate stages in the life cycle at 32°C. in 45, 60, 75, and 90 per cent humidities; based on results obtained from 6 beetles in each stage.*

Beetle Stage	Percent of Humidity			
	45	60	75	90
Eggs	7	6	4	5
Larvæ	22	20	16	19
Pupæ	9	9	9	9
Adults**	12	14	14	16

*Length of each stage was considered as being the elapsed time until the first emergence in days.
**Average only.

PERCENTAGE OF VIABLE EGGS UNDER CONTROLLED CONDITIONS

Experiments to determine the percentage of viable eggs were conducted. These were carried on at 32°C. and in 45, 60, 75, and 90 per cent humidities. They were started with 100 eggs at each humidity. All eggs had been laid within the 12 hour period before being used.

In determining the viability of the eggs, 4 desiccators were employed, one for each humidity. The eggs were kept in low dishes. The whole experiment was conducted in one of the boxes regulated to maintain a temperature of 32°C. Daily examination of the eggs gave the results shown in Table III and in Figures 8 and 9.

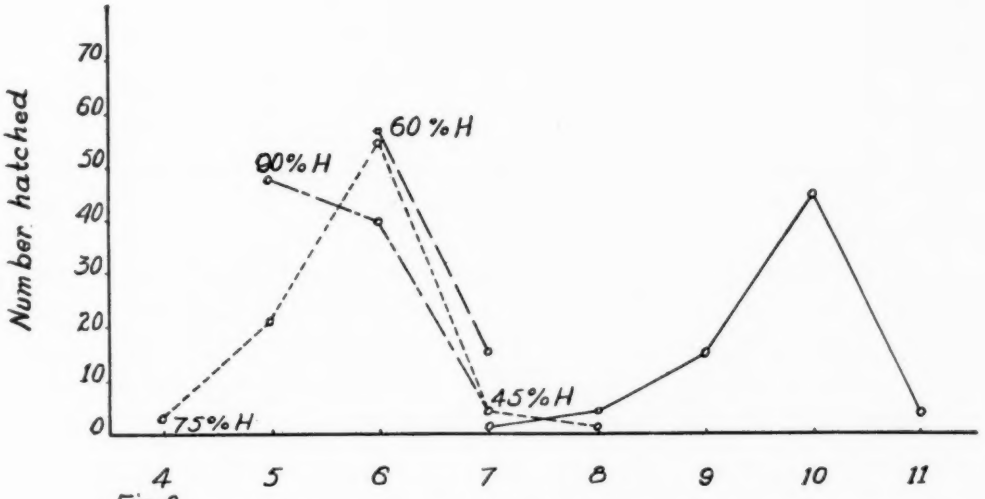

Fig. 8
The incubation period by days, at 32°C and different humidities, based on 100 eggs

The results of this experiment based on 100 eggs in each humidity and at 32°C. show: (1) that eggs of the tobacco beetle are affected by humidity during the incubation period; (2) eggs begin to hatch in 4 days in 75 per cent humidity; (3) lower humidities retard the rate of incubation; (4) the number of eggs hatched is determined by the humidity; (5) only a small per cent fail to hatch at optimum humidity, while 30 per cent fail to hatch in 45 per cent.

The experiment to determine the total percentage of beetles which completed the cycle was conducted in the apparatus shown in Fig. 2. Tobacco and yeast served as food. Table IV and Fig. 10 show the results obtained from the yeast. The beetles were counted daily and removed from the experimental jars as the life cycle was completed. Fewer beetles completed the life cycle in tobacco than in yeast. When tobacco was used as the food 54 beetles came to maturity in 75 per cent humidity; 46 in 60; and 42 in 45.

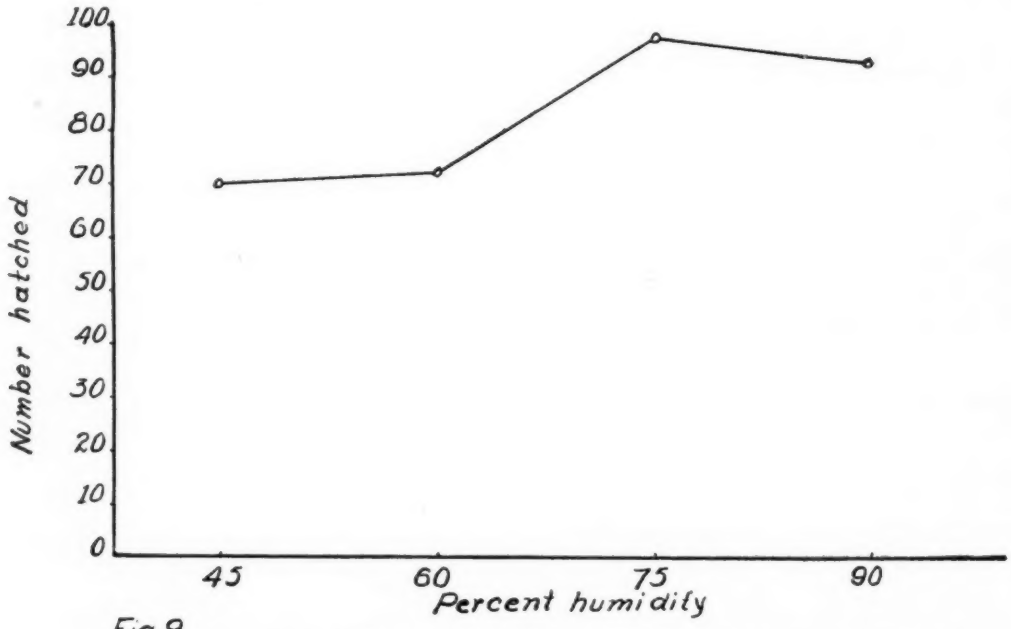

Fig. 9
Showing percent of eggs hatched at 32°C and different humidities based on 100 eggs

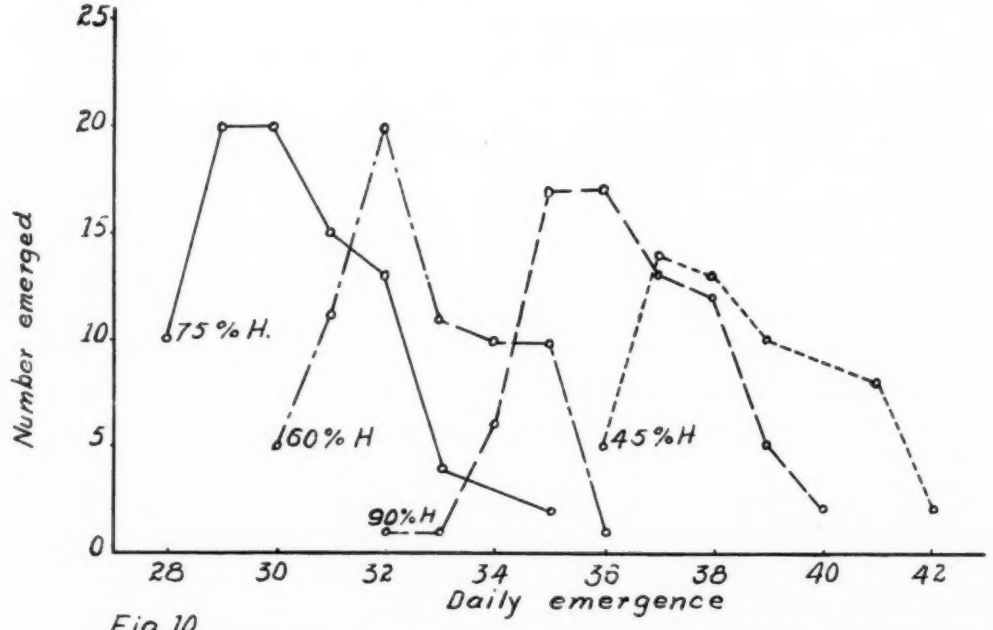

Fig 10
Showing viability percentage of the beetle life cycle at 32°C and different humidities based on results obtained from 100 eggs at each humidity

This experiment with 100 eggs, at 32°C. and in humidities of 45, 60, 75, and 90 per cent shows: (1) that more beetles complete their life cycle in yeast than in tobacco; (2) that there is a variation of from 7 to 8 days in the life cycle in the same humidity and at the same temperature; (3) the optimum humidity is 75 per cent as judged by length of life cycle and per cent of beetles produced; and (4) humidities lower than 75 per cent cause the life cycle to be lengthened.

TABLE III. *The incubation period by days at 32°C. and 45, 60, 75, and 90 per cent of humidity based on results obtained from 100 eggs.*

Humidity	Daily record of eggs hatched								No. hatched
Percent	4th	5th	6th	7th	8th	9th	10th	11th	Percent
45	0	0	0	1	4	15	46	4	70
60	0	0	57	15	0	0	0	0	72
75	3	21	65	4	1	0	0	0	94
90	0	48	40	4	0	0	0	0	92

MATING

To prove whether tobacco beetles mate more than once, 10 pairs were caught mating. Each pair of individuals was placed in a two ounce glass jar with a cloth top. The insects were observed from time to time. On two separate days the same adults were observed in the act of copulation, which proves that tobacco beetles will mate more than once.

TABLE IV. *Percentage of the beetles which completed their life cycles at 32°C. in 45, 60, 75, and 90 per cent humidity. Experiment started with 100 eggs.*

Humidity percent	Record of daily emergence															No. emerged, percent
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
45	0	0	0	0	0	0	0	0	5	14	13	10	9	8	2	61
60	0	0	5	11	20	11	10	10	1	0	0	0	0	0	0	68
75	10	20	20	15	13	4	3	2	0	0	0	0	0	0	0	87
90	0	0	0	0	1	1	6	17	17	13	12	5	2	0	0	74

SEX RATIOS

In order to determine the average number of males per female, 100 tobacco beetles were caught as they emerged from the pupal stage and kept together for three days. This was done so that the females would be fertilized. At the end of three days the beetles were separated. Each individual was placed in a small glass vial with sufficient folded tobacco for food and shelter. After another three days all of the jars were carefully examined for eggs. Those in which eggs were found were labeled females and the others were denoted the males. In previous experiments it has been found

that a female is very rarely found that does not lay eggs during the first six days of her life. There were 54 vials found with eggs and 46 had none. These results, with 100 individuals, show a ratio of 46 males to 54 females.

AVERAGE NUMBER OF EGGS LAID BY A FEMALE BEETLE UNDER CONTROLLED CONDITIONS

Preliminary experiments showed that the tobacco beetle copulates more than once, hence the fact that adults are found copulating does not mean that they have just emerged and are getting ready to lay. To avoid old beetles which would produce few eggs, a large stock was reared. This gave a good number of adults on the first day of the emergence. These were taken

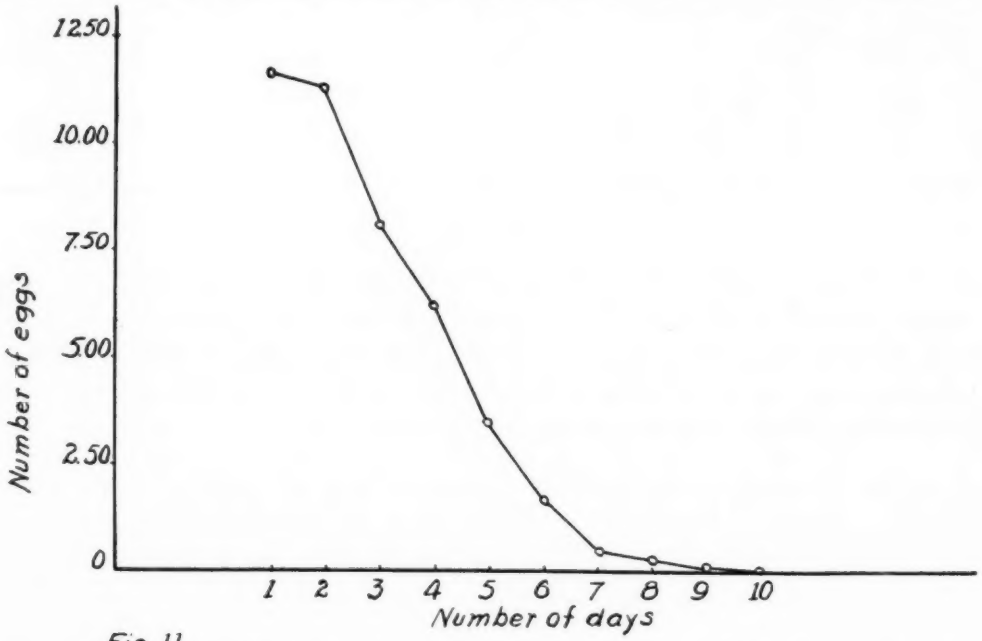

Fig. 11
Showing the average number of eggs laid by a single female beetle each day under controlled conditions

from the stock jars and placed in several containers. By watching the insects for a number of hours, one hundred pairs were caught copulating. This then made all beetles used in the experiment the same age—*i.e.*, they emerged and mated within the same twenty-four hour period. Each pair of adults was placed in a small glass vial 20 mm. wide by 80 mm. long. A piece of cloth held on by a rubber band served as top. In each vial a small piece of tobacco was placed on which the female might lay her eggs.

In order to avoid any outside factors that would detract from the accuracy of the experiment, one hundred vials were now placed in a large desiccator jar which contained sulphuric acid which was diluted to produce a humidity of 75 per cent. The jar was kept in an oven at 32°C. These conditions, namely, 32°C. and 75 per cent humidity were maintained throughout. The

experiment was carefully gone over at the end of every twenty-four hours and the results tabulated. Each day the tobacco was renewed. The stock from which the new tobacco was taken, day by day, was kept in the desiccator along with the beetles. This was done in order that the moisture would always be constant throughout the experiment. Table V shows the number of eggs laid each day, total number laid per pair, total average per day, and total average.

The average number of eggs laid by a single female based on the record of 100 pairs of beetles was 43.57. Most of the eggs were laid during the first 6 days. The largest number (1166) was laid on the first day, while on the second day (1129) were laid. The third day shows a falling off of 286, making a total of 843 eggs for that day. Fig. 11 shows the average by days for all the beetles. It is apparent in the table that the number of eggs laid by a single female varied considerably. This has been noticed in other similar experiments in which eggs have been counted daily. The smallest number of eggs laid by a single female was one egg, while the largest number was 95. Only three females laid fewer than 10 eggs, while 6 laid between 10 and 20. One beetle passed the 90 mark, two laid 81 each, while 7 laid totals between 70 and 80.

For the purpose of determining what effect smaller groups would have on the average number of eggs per day the record shown in Table V was divided into 20 groups of 5 beetles and the averages taken. The first group consisted of the first five beetles, the second of the next five, etc. The lowest group gave 30 eggs per beetle, while the highest made a total of 59. Seven groups gave totals between 30 and 40, seven between 40 and 50, while 6 were between 50 and 60. In the latter group there were averages of 50, 51, three of 52, and 59.

This experiment shows: (1) that the average number of eggs laid by a female tobacco beetle in her entire life-time was 43.57; (2) the greatest number of eggs were laid during the first 6 days; (3) there was a wide variation in number of eggs laid by individual beetles (1-93).

AVERAGE NUMBER OF EGGS LAID BY A BEETLE UNDER ROOM CONDITIONS

In determining the average number of eggs laid by a single beetle under room conditions, 66 pairs of beetles were obtained from the tobacco storages. All beetles were caught copulating so that each female would be fertile. The females were placed in small glass vials, 20 mm. by 80 mm., with a cloth top help on by rubber bands. The vials were left at room temperature and a daily count of eggs was made. From this number of beetles an average of 24 eggs per female was obtained. Thus the oviposition under room conditions of beetles caught in a warehouse, even though they were caught mating, resulted in few eggs per individual.

TABLE V. *An egg-record obtained by counting daily the number of eggs laid by one hundred fertile female beetles, at 32°C. and 75 per cent humidity.*

Beetle pairs	Daily record										Total per pair
	1	2	3	4	5	6	7	8	9	10	
1	6	11	18	6	4	1	0	0	0	0	46
2	0	0	0	25	12	11	8	4	1	0	61
3	12	20	8	7	3	0	0	0	0	0	50
4	15	11	5	1	1	5	0	0	0	0	38
5	14	25	15	3	0	0	0	0	0	0	57
6	9	11	5	0	0	0	0	0	0	0	25
7	14	21	15	8	2	10	4	0	0	0	74
8	3	8	6	1	0	0	0	0	0	0	18
9	21	19	8	5	6	2	0	0	0	0	61
10	22	15	4	6	1	0	0	0	0	0	48
11	18	9	17	3	3	0	0	0	0	0	50
12	10	19	4	7	5	2	0	0	0	0	47
13	14	10	9	5	4	0	0	0	0	0	42
14	2	16	6	0	8	4	0	1	0	0	37
15	16	10	14	0	0	0	0	0	0	0	40
16	0	2	12	3	1	0	1	5	0	0	24
17	1	19	18	15	10	3	3	0	0	0	69
18	10	8	11	7	2	4	0	0	0	0	42
19	10	2	13	5	3	1	0	0	0	0	34
20	15	17	10	6	4	0	0	0	0	0	52
21	21	14	5	7	8	0	0	0	0	0	55
22	3	11	5	5	4	0	0	0	0	0	28
23	22	12	8	3	3	3	1	0	0	0	52
24	12	25	7	7	7	3	0	0	0	0	61
25	32	18	10	0	2	0	0	0	0	0	62
26	28	10	9	14	2	2	2	0	0	0	67
27	22	12	13	12	2	0	0	0	0	0	61
28	14	10	1	4	1	0	0	0	0	0	30
29	4	9	9	0	0	0	0	0	0	0	22
30	17	10	9	12	3	1	0	0	0	0	52
31	20	20	16	11	4	2	0	1	1	0	75
32	19	27	16	11	8	0	0	0	0	0	81
33	15	6	3	3	13	4	2	4	0	0	50
34	5	13	7	5	2	1	0	0	0	0	33
35	12	4	0	0	0	0	0	0	0	0	16
36	10	8	13	13	0	2	2	0	0	0	48
37	2	25	17	13	13	2	0	0	0	0	72
38	0	0	7	2	13	12	7	4	0	0	45
39	0	0	1	0	0	0	0	0	0	0	1
40	11	18	23	10	3	0	0	0	0	0	65
41	15	8	3	1	0	0	0	0	0	0	27
42	18	0	1	0	1	0	0	2	1	1	24
43	23	14	15	8	17	3	1	0	0	0	81
44	9	17	6	0	0	0	0	0	0	0	32
45	25	30	11	19	6	2	1	1	0	0	95
46	4	10	4	2	1	1	0	0	0	0	22
47	10	9	7	13	2	11	0	0	0	0	52
48	9	9	8	2	1	0	0	0	0	0	29
49	1	11	17	0	0	0	0	0	0	0	29
50	8	15	7	13	2	1	0	0	0	0	46
51	7	7	13	6	1	0	0	0	0	0	34
52	4	9	12	0	0	0	0	0	0	0	25
53	0	13	9	6	0	0	0	0	0	0	28
54	16	17	15	8	8	2	1	0	0	0	67
55	10	8	6	18	2	0	0	0	0	0	44
56	7	18	6	8	0	1	0	0	0	0	40
57	12	5	2	5	0	0	0	0	0	0	24

TABLE V. *(Continued)*

Beetle pairs	Daily record										Total per pair
	1	2	3	4	5	6	7	8	9	10	
58	2	0	0	1	0	0	0	0	0	0	3
59	25	15	4	1	0	0	0	0	0	0	45
60	12	9	7	7	0	0	0	0	0	0	35
61	10	8	2	7	0	0	0	0	0	0	27
62	7	8	6	3	10	1	0	0	0	0	35
63	20	24	12	10	6	3	0	0	0	0	75
64	20	13	12	5	3	0	0	0	0	0	53
65	12	8	16	7	3	0	0	0	0	0	46
66	5	2	1	0	0	0	0	0	0	0	8
67	8	11	7	0	0	1	0	0	0	0	27
68	13	22	11	12	7	4	0	0	0	0	69
69	8	13	8	0	0	1	0	0	0	0	30
70	14	10	4	4	20	0	0	0	0	0	52
71	5	7	9	4	0	0	0	0	0	0	25
72	11	10	9	7	6	0	0	0	0	0	43
73	15	5	19	5	2	2	0	0	0	0	48
74	4	11	3	3	1	0	0	0	0	0	22
75	14	13	12	4	0	0	0	0	0	0	43
76	15	17	3	0	0	0	0	0	0	0	35
77	12	16	14	6	6	0	0	0	0	0	54
78	4	3	6	9	3	6	1	0	0	0	32
79	12	5	0	1	0	0	0	0	0	0	18
80	14	11	8	3	0	0	0	0	0	0	36
81	10	5	8	0	3	1	0	0	0	0	27
82	6	4	3	0	0	0	0	0	0	0	13
83	10	19	9	5	4	0	0	0	0	0	47
84	6	3	5	0	4	0	0	0	0	0	18
85	37	17	12	4	5	0	0	0	0	0	75
86	20	12	23	7	4	3	1	0	0	0	70
87	23	16	15	3	0	0	0	0	0	0	57
88	12	3	5	18	3	6	7	1	0	0	55
89	8	13	7	12	8	4	1	0	0	0	53
90	0	15	9	0	0	0	0	0	0	0	24
91	10	2	5	8	3	1	0	0	0	0	29
92	19	13	2	14	6	5	2	0	0	0	61
93	0	0	4	7	2	7	1	1	0	0	22
94	3	3	4	1	0	1	0	0	0	0	12
95	0	3	2	35	5	3	0	0	0	0	48
96	17	13	15	12	2	3	1	0	0	0	63
97	18	16	3	1	0	0	0	0	0	0	38
98	15	8	3	16	6	14	0	0	0	0	62
99	19	1	14	19	17	3	1	0	0	0	74
100	12	17	3	16	3	4	2	1	0	0	58
Total.....	1166	1129	843	631	340	169	50	25	3	1	4357
Average ..	11.66	11.29	8.43	6.31	3.40	1.69	.50	.25	.03	.01	43.57

THE EFFECT OF NUMBERS OF INDIVIDUALS ON THE OVIPOSITION OF BEETLES

A series of experiments were conducted to show what effect numbers of individuals would have on oviposition. These were all conducted under similar conditions, at room humidity and temperature. Four-ounce jars with cloth tops were used as the containers. The same amount of tobacco (Grade

D. E. N. C.) was placed in each jar. The experiment was run in two sets, of 1, 2, 3, 4, 5, and 6 pairs of beetles. The pairs were all caught mating, which insured that each female was fertile. The experiments were carefully gone over each day. All eggs were counted and removed. The results obtained from both sets were consolidated and are shown in Table VI. In this connection an experiment was conducted to determine whether or not females would lay without the males. The conditions were the same as described above. Two jars were used. In one 10 males and 5 females were placed, and in another only 5 females. At the end of the period of oviposition, 129 eggs or an average of 26 to each female beetle had been counted in the first jar; in the second jar 136 eggs were obtained, or an average of 27 per female. Eggs were never obtained from beetles kept in jars without food. An experiment was carried out to determine if eggs were destroyed, or if there was a total absence of oviposition under such conditions. This experiment was conducted in duplicate. Twelve pairs of beetles were selected, allowed to lay, and 24 of the eggs were placed in an empty jar with the twelve pairs of beetles. The eggs were examined from day to day. None of them were ever eaten or otherwise damaged but the original number of eggs was not increased.

These results show: (1) that as many as twelve pairs of beetles to a 4 ounce jar will not materially alter the average number of eggs laid by a beetle; (2) after the female has been fertilized the presence or absence of the male beetle does not affect oviposition; (3) beetles do not eat their eggs nor destroy them.

TABLE VI. *The effect of numbers of individuals on oviposition of beetles. The experiments were carried out in two sets, of 1, 2, 3, 4, 5, and 6 pairs each. These are combined and an average of the whole is given.*

No. of pairs	Daily record of oviposition								Total	Average per pair
	1	2	3	4	5	6	7	8		
2	8	8	12	14	8	2	0	0	52	26.0
4	40	8	16	28	15	3	2	0	112	28.0
6	65	15	40	27	7	4	0	0	158	26.0
8	75	24	48	26	19	32	7	5	236	29.5
10	41	34	41	23	9	10	5	2	165	16.5
12	89	58	53	17	21	37	17	0	292	24.0

EFFECT OF THE PRESENCE OR ABSENCE OF PARTICULAR MATERIALS ON OVIPOSITION

In an effort to determine what effect, if any, the presence or absence of particular materials have on egg laying of tobacco beetles experiments were performed in which only tobacco was used as the medium to induce the insects to lay eggs. Four groups each consisting of twenty pairs of adults were used. These will be referred to as groups (a), (b), (c), and (d). 1, 2, 3, 4, and 10 pairs were placed in separate jars. The insects were caught in the to-

bacco storage warehouses just previous to the experiments, all in the act of copulation. Group (a) consisted of adults that were put with tobacco on alternate days. The first day tobacco was supplied; the next day it was removed and the beetles were kept thereafter in empty jars. Group (b) was supplied with tobacco during the first three days, and at the end of that time was kept in empty jars. Group (c) was kept in empty jars during the first three days and then supplied with tobacco which remained until all beetles were dead. Group (d) was run as a control and continually supplied with tobacco.

TABLE VII. *Showing the effect of the presence or absence of particular materials on oviposition of the tobacco beetle.*

Group	Pairs of Beetles	Daily record of oviposition									Total	Average per pair
		1	2	3	4	5	6	7	8	9		
(a)	1	11	00	22	00	18	00	7	00	00	58	58
Tobacco on	2	8	00	15	00	2	00	0	00	00	25	12.5
alternate days	3	18	00	19	00	5	00	2	00	00	44	15
	4	34	00	45	00	4	00	0	00	00	83	21
	10	50	00	65	00	28	00	4	00	00	147	15
(b)	1	4	11	7	00	00	00	0	00	00	22	22
Tobacco first three	2	2*	5	11	00	00	00	0	00	00	18	18
days	3	25	32	14	00	00	00	0	00	00	71	24
	4	45	27	34	00	00	00	0	00	00	106	26
	10	105	88	55	00	00	00	0	00	00	248	25
(c)	1	00	00	00	00*	00	00	0	00	00	00	00
Tobacco after first	2	00	00	00	12	7	1	0	00	00	20	10
three days	3	00	00	00	1	1	1	0	00	00	3	1
	4	00	00	00	1	1	1	1	00	00	4	1
	10	00	00	00	30	5	1	3	00	00	39	4
(d)	1	13	11	9	00	00	00	0	00	00	33	33
Control tobacco	2	4	6	6	6	8	1	0	00	00	31	15
every day	3	35	14	9	14	3	2	0	00	00	77	25
	4	13	47	31	22	00	00	0	00	00	113	28
	10	10	40	20	21	6	4	0	00	00	101	10

* One beetle was found dead.

The experiments were carried on under controlled conditions at a constant temperature of 32°C. and in a humidity of 75 per cent. The containers consisted of two ounce, and four ounce glass jars, and the 10 pair set was run in tall glass dishes. All the jars were covered with cloth. The test material used in each jar was of the same type—the best grade of Eastern North Carolina tobacco. Each experiment was examined carefully at the end of every twenty-four hours, and results tabulated.

Table VII shows egg laying by days and the average per pair for the different groups. In Group (a), in which the beetles were kept in tobacco on alternate days, eggs were laid every day that tobacco was present and none were deposited on the days when there was no tobacco. This continued until

the insects died. The total average of eggs per pair of adults for this group was 24.3, which is slightly more than the average per pair for Group (d) which was run as a control. This probably does not mean that beetles will actually lay more eggs under such conditions, but that they will average as many eggs laying on alternate days as when laying every day. In Group (b) the beetles laid eggs every day for the first three days and then ceased altogether when the tobacco was removed. The total average for this group was 23 eggs per pair of adults. In Group (c) there was a total absence of eggs during the first three days and then each set laid some eggs. In Group (d) egg laying occurred normally for beetles under such conditions.

The results of these experiments show: (1) that females will not lay in the absence of materials upon which to lay; (2) if beetles are kept without material upon which to lay for the first three days after they copulate, the total number of eggs laid will be considerably reduced.

EFFECT OF FERTILIZATION ON OVIPOSITION

Experiments were conducted to determine whether unfertilized females of the tobacco beetle would oviposit. One-hundred pupae were placed in separate vials (20 mm. by 80 mm.) upon emerging as adult beetles were changed into two ounce jars, each of which contained a small piece of folded tobacco. All of the jars were kept at room humidity and temperature.

Group (a) consisting of Numbers 1, 2, 3, and 4 were females. At the end of the fourth day they were put together. On the ninth day no eggs had been laid and male 57 was then introduced into the jar with them. Copulation began at once and the next day 35 eggs were found. This group laid a total of 204 eggs within the next ten days. Females 5, 6, 7, and 8 constituted group (b). They were put together on the third day. Eggs were not laid by the end of the tenth day and so males number 48 and 69 were placed with them. On the eleventh day eggs were found. Oviposition ended in this group on the twentieth day, after a total of 194 eggs had been laid. Group (c) consisting of numbers 9 to 18, were combined at the end of the third day. Mating was observed at once. A total of 433 eggs were obtained from the group. Group (d), numbers 19 to 41, were combined in the same manner as (c) and eggs were laid only after the beetles had been put together. Numbers 42 to 58 (except 48 and 57), constituting group (c), were kept separate for the first 6 days. No eggs were laid during this time. After these beetles were allowed to mate 348 eggs were obtained. Group (f), numbers 59 to 70 (except 69), were kept separate the whole life time. In no instance were any eggs found. Group (g), numbers 71 to 76 were combined as soon as they emerged. There were no eggs on the first day, but 6 on the second. The oviposition period lasted for 12 days. A total of 175 eggs were deposited during this period. Group (h), numbers 77 to 90, were treated in the same manner as Group (f) and with the same results. Only 90 out of the original 100 pupae emerged.

Table VIII gives a summary of the experiments. The results show: (1) that unfertilized females do not oviposit; (2) copulation occurs at once when males and females that have been kept separate are placed together; (3) oviposition follows copulation within 24 hours; (4) when mating does not occur at once after emergence oviposition will be delayed in proportion to the amount of time elapsed (not over 10 days) in the premating period.

TABLE VIII. *The effect of fertilization on oviposition.*

Group	Beetle numbers	Number in group	Day when combined	Day oviposition		Total eggs
				Began	Ended	
a	1-4, 57	5	4 (57 on 9)	10	19	204
b	5-8, 48, 69	6	3 (48 and 69 on 10)	11	20	194
c	9-18	10	3	4	16	433
d	19-41	23	3	4	13	700
e	42-47, 49-56, 58	15	6	7	17	348
f	59-68, 70	11	Not combined	None	None	None
g	71-76	6	As soon as emerged	2	13	175
h	77-90	14	Not combined	None	None	None

THE EFFECT OF PHYSICAL FORM OF MATERIALS ON OVIPOSITION

In an effort to determine what effect physical form alone exerts on the oviposition of the tobacco beetle, a number of substances were tested. All of these experiments were conducted under room conditions. Tall glass dishes covered with cloth held on by rubber bands served as containers. Various substances induced the female to oviposit. The filter paper used in this experiment and in all others in which filter paper is mentioned was Whatman's No. 40. Besides filter paper other substances tried were iron filings, wool, glass wool, potassium dichromate, sawdust, sand, and glass. Table IX shows the results obtained when iron filings, wool, glass wool, potassium dichromate, and filter paper were used. None of these materials were sized in any manner. Twenty-five beetles were placed in small amounts of each material, and the eggs laid were carefully removed and counted each day. Sand and glass were also used as stimuli for oviposition. These materials were graded with sieves made from standard bolting cloth.* Table X shows the results obtained. Sawdust was graded in the same manner into twelve different sizes. In this case 12 beetles were used with each size of particle. Tobacco was used as a control in conjunction with the sawdust. Results obtained from this experiment are shown in Table XI.

The results of these experiments show: (1) that the tobacco beetle will oviposit on various organic and inorganic substances which are quite unrelated to tobacco; (2) the size of particles in materials available is a determining

* Numbers of the standard bolting cloth sieves correspond to the following number of meshes to the inch: 0000—18, 000—22, 00—30, 0—40, 1—48, 2—52, 3—56, 4—60, 5—64, 6—72, 7—80, 8—84, 9—96, 10—106, 11—116, 12—124, 13—130, 14—140, 15—148, 16—156.

TABLE IX. *Oviposition as induced by several substances, none of which were graded in regard to size. Results shown were obtained from 25 beetles in each type of material.*

Substance	Daily record of oviposition					
	1	2	3	4	5	6
Iron filings	22	35	30	16	2	0
Wool	9	10	3	1	1	0
Glass wool	22	3	6	4	13	0
Potassium dichromate	6	4	24	0	0	0
Filter paper	0	0	0	0	0	0

TABLE X. *Sand and glass as stimuli for oviposition. Materials were sized, and 25 beetles were placed with each size of particles in each set.*

Size of particles*	Daily record of oviposition								
	Sand						Glass		
	1	2	3	4	5	6	1	2	3
0000	0	0	0	0	0	0	0	0	0
000	0	2	6	8	0	1	1	2	2
00	0	2	0	1	3	0	0	0	0
0	0	0	1	2	1	1	0	3	11
1	0	1	0	2	0	0	2	4	2
2	0	2	0	0	1	2	11	2	0
3	2	0	0	0	6	0	10	6	8

*"Excelsior Testing Sieve"; Hammond-Homberberger Co., Minneapolis, Minn.

TABLE XI. *Sawdust and tobacco as stimuli for oviposition. Materials were graded into 12 sizes; 12 beetles in each size.*

Size of particle	Daily record of oviposition					
	Sawdust				Tobacco	
	1	2	3	4	5	6
0000	0	0	0	0	18	6
000	24	15	8	3	110	31
00	21	12	13	7	31	8
0	7	19	5	6	53	11
1	0	3	2	0	17	3
2	22	10	2	0	23	3
3	5	7	16	3	84	70
4	1	4	13	2	55	24
5	2	0	2	3	57	7
6	1	0*				
7	1	0	0	0	21	3
8	1	1	0*			
9	9	6	1	0	27	4
10	20	9	5	3	24	12
11	6	13	8	9	14	0
12	7	2	1	0	53	18

*Beetles cut hole through cloth top and escaped.

factor for oviposition; (3) oviposition does not occur on coarser materials than size 0000; (4) physical form influences oviposition but not as completely as when tobacco is used; (5) beetles will not lay on smooth filter paper, unless it is divided into small pieces, they then lay at the edges where the paper is rough.

THE EFFECT OF ODORS ON OVIPOSITION

Experiments were conducted to determine the potency of odors as stimuli for oviposition and a number of different substances were tried. Positive results were obtained from hot water extracts of tobacco and from weak solutions of nicotine. The same general conditions were observed as in the experiments on physical form. Since filter paper will not of itself cause oviposition, this substance was chosen as the medium to be saturated with the odorous substance for each test.

The first experiment was performed with hot water extracts of tobacco. The extract was made by steeping 40 grams of tobacco in 400 cc. of hot water for 15 minutes. The extract was aerated before use. Filter paper was soaked in this substance and then thoroughly dried. The beetles would not oviposit on the paper if it was even slightly damp. Twelve pairs of beetles were placed in each stender dish. The filter paper carrying the extract was renewed each day. Care was exercised to prevent tearing or roughening the paper in any manner as this might induce oviposition as a result of physical stimuli. The beetles were observed for a period of 6 days. Eggs were found on each day of the experiment, when the paper had been thoroughly dried, but each individual did not lay consistently. Two controls were run at the same time. One contained filter paper which had been moistened in distilled water and no eggs were ever obtained. The other control contained tobacco leaf. Eggs laid during the experiment are summarized in Table XII.

Preliminary experiments with nicotine solution showed that the beetles would not oviposit on materials infiltrated with solutions stronger than one per cent. Two to three per cent solutions affected the beetles injuriously, while stronger solutions soon caused death, even though containing jars were covered with cloth. Filter paper was the medium used for carrying the solutions to be tested. Ten percentages (.1 to 1.0) were used. Six drops of each strength was applied to a piece of filter paper. The paper was then dried and placed with beetles for 24 hours. Six pairs of beetles were placed in each jar. Filter paper and distilled water constituted the first control and tobacco the second. Results of this experiment are shown in Table XIII. Corn sugar, cane sugar, gelatine, fats, egg albumen, and blood serum were tried in various percentages. None of these substances caused the beetle to oviposit.

These experiments show the following facts: (1) that the presence of odorous substances will cause the tobacco beetle to oviposit to a limited extent; (2) hot water extracts of tobacco are more efficient than nicotine solutions,

which are rather weak stimuli; (3) in no case does oviposition when induced by odorous substances applied to filter paper approach the average number of eggs laid by beetles on tobacco.

TABLE XII. *Oviposition as induced by filter paper soaked with hot water extract of tobacco, results based on record obtained from 12 pairs of beetles to each jar.*

Jar number	Daily record of oviposition					
	1	2	3	4	5	6
1	0*	12	11	1	9	5
2	0*	3	10	0	5	0
3	0*	3	5	1	0	0
4	0*	15	7	0	2	1
5	0*	2	7	0	0	0
6	0*	10	10	1	1	0
Control No. 1 Dist. H_2O	0	0	0	0	0	0
Control No. 2 tobacco	10	46	34	21	16	7

*Filter paper was not thoroughly dried before use on this day.

TABLE XIII. *Oviposition as induced by the odors from 6 drops of nicotine solution applied to filter paper. Six pairs of beetles were used for each test.*

Percentage of nicotine solution	Daily record of oviposition					
	1	2	3	4	5	6
0.1	0	0	0	0	0	0
0.2	0	1	0	2	3	0
0.3	3	0	4	1	0	1
0.4	2	0	1	2	2	0
0.5	0	0	0	0	0	1
0.6	0	0	1	0	0	1
0.7	0	0	0	0	0	2
0.8	0	0	0	1	0	0
0.9	0	1	0	0	0	0
1.0	0	0	0	0	0	0
Distilled water	0	0	0	0	0	0
Tobacco	12	30	70	14	16	20

COMPARISON OF THE NUMBER OF EGGS LAID ON VARIOUS SUBSTANCES

An experiment was conducted to compare the number of eggs laid on 6 different types of tobacco and 3 kinds of furniture materials. The following types of tobacco were experimented with: (1) best grade of Eastern North Carolina tobacco, 1928 crop; (2) and T. South Carolina, 1927 crop; (3) Turkish tobacco—Cavalla No. 4, Smyrna No. 2, Smyrna No. 4, Xanthia No. 3, 1926 crop. The furniture materials were furnished by The Kroshler Mfg. Co., Naperville, Ill. They consisted of Spanish moss, palm fiber, and flax tow.

This experiment was carried on at 32°C. and in 75 per cent humidity. Two-ounce jars covered with cloth were used as containers, and a small amount of each type of material was introduced into each. Care was used in

selecting the beetles. Five pairs caught mating immediately after emerging were placed in the jars. The results of the experiment are shown in Table XIV and Figure 12. It is apparent that the tobacco beetle lays fewer eggs on

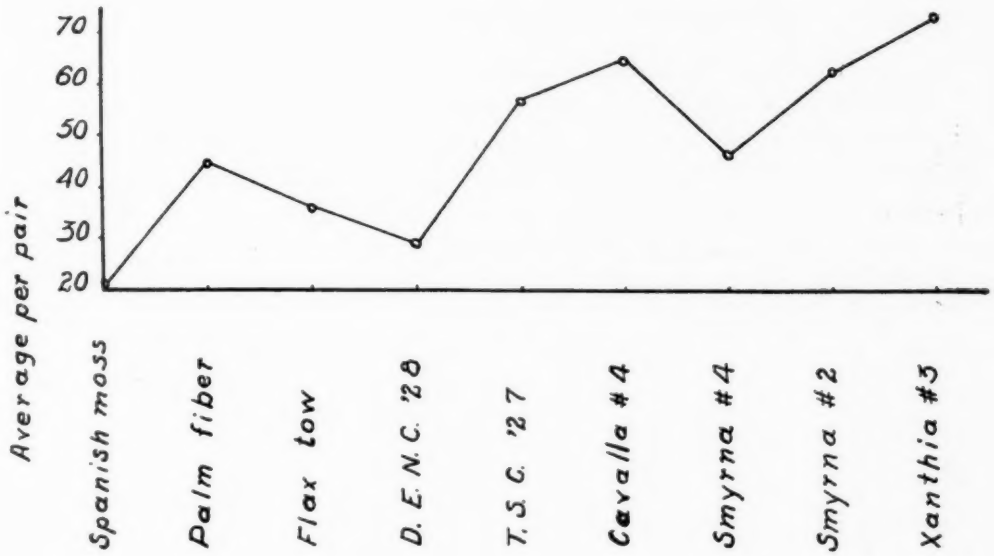

Fig. 12
Oviposition in different substances, based on 5 pairs of beetles

the furniture materials than on the tobacco. The Turkish types of tobacco are the most efficient inducers. These results are based on the oviposition records of only 5 female beetles with each food substance. Individual variations among beetles, as shown in the experiments concerning the average number of eggs per beetle, indicate that conclusive experimental proof would have to come from larger numbers of beetles in each group.

TABLE XIV. *Comparison of number of eggs laid by beetles in different substances. The record was obtained from 5 pairs of beetles in each material.*

Substance	Daily record of oviposition								Total	Average per pair
	1	2	3	4	5	6	7	8		
Spanish moss	26	27	34	6	1	5	4	2	105	21
Palm fiber	53	77	42	27	12	5	3	0	219	45
Flax tow	61	30	42	33	13	1	0	0	180	36
Tobacco:										
E. N. C. 28	33	40	33	18	7	5	8	0	144	29
T. S. C. 27	95	46	55	51	21	9	5	4	286	57
Cavalla No. 4	60	124	80	37	12	8	3	2	326	65
Smyrna No. 4	67	66	40	33	21	3	1	0	231	46
Smyrna No. 2	112	64	64	51	12	3	3	2	311	62
Xanthia No. 3	82	81	108	63	26	4	2	0	366	73

FOOD PREFERENCES OF THE TOBACCO BEETLE

Food preferences of the beetle were studied from several standpoints: (1) size of tobacco particle; (2) variations in length of the life cycle when different kinds of tobacco were used as food; (3) the reactions of beetles to the odors of different kinds of substances.

SIZE OF TOBACCO PARTICLE

In order to determine what effect different sizes of tobacco particles exert on the length of the life cycle, tobacco was ground and sieved into twelve different sizes. The type of tobacco used was the best grade of Eastern North Carolina tobacco, 1927 crop. The size of the particles into which the tobacco was graded ran from 00000 to 7 (standard bolting cloth sieves; see p. 361.

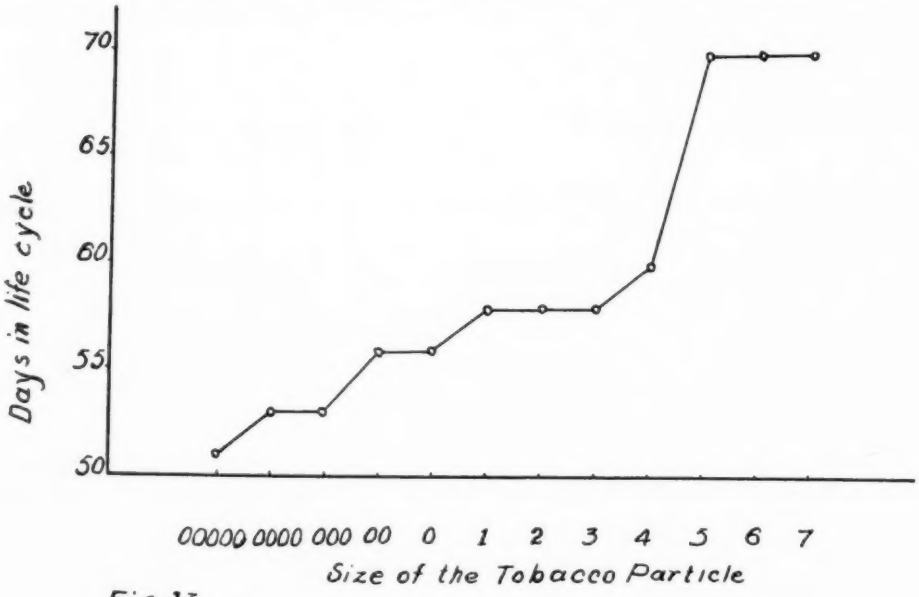

Fig. 13
Showing the effect of the size of tobacco particles on the Length of the beetle life cycle

The experiment was carried on at room temperature and humidity. Tall glass stender dishes with cloth covers were used as containers, and four grams of tobacco were placed in each. The beetles used were adults caught mating and four pairs were placed in each jar. The experiment was run in duplicate. Table XV and Figure 13 show the results obtained. The length of life cycle varied from 51 to 70 days, the shortest period in the coarsest tobacco, and the longest in the finest size of particle.

The results from this experiment show: (1) that when tobacco is ground the size of particle is a factor in the length of the life cycle of the beetle; (2) the coarser particles are associated with a shorter life-cycle and are therefore believed to be most favorable, while the finer particles increase length of the cycle.

TABLE XV. *The effect of different sizes of tobacco particles on the length of the beetle life cycle. Four pairs of beetles, caught mating, were used in each size of tobacco. Experiments were run in duplicate. Tobacco type, 129.*

Size of particles	00000	0000	000	00	0	1	2	3	4	5	6	7
Days in life cycle.....	51	53	53	56	56	58	58	58	60	70	70	70

DIFFERENT TYPES OF TOBACCO

Twelve different types of tobacco were chosen. The same conditions were observed as in the last experiments, the only difference being that various kinds of tobacco were placed in the jars instead of different sizes of particles. The types of tobacco were ground but not sieved. The different types used in this experiment are referred to by number, all of which are explained in Table XVII; except No. 129, which was the best grade of Eastern North Carolina tobacco, 1927 crop.

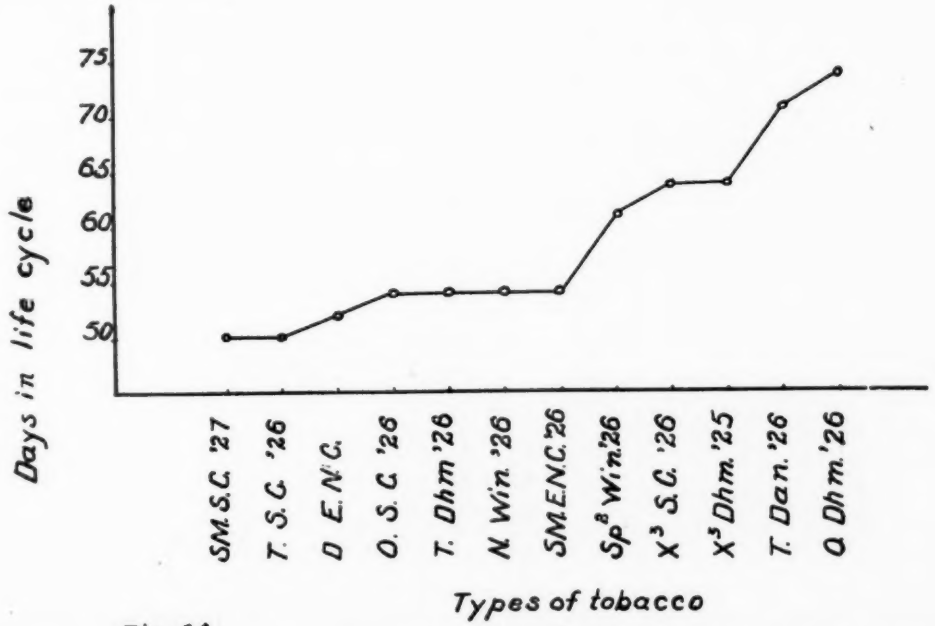

Fig. 14
Showing the effect of different types of tobacco on the length of the life cycle of the beetle.

Table XVI and Fig. 14 show the results obtained. The tobacco is classified according to the length of life cycle. This period varied from 50 to 74 days. Using the length of life cycle as a criterion, the tobacco classified as the "better grades" is associated with a shorter life cycle for the beetle. Number 95 and 96 were optimum among those tried. It is interesting to note that 95 is described as being, "excellent, well aged, good smoking tobacco." On the other hand in number 99 the life cycle was 24 days longer. This type is described as being "leafy and heavy bodied"—an inferior grade.

TABLE XVI. *The length of the life cycle of the tobacco beetle as determined by different types of tobacco. Four pairs of beetles, caught mating were used in each type of tobacco. Experiments were run in duplicate.*

Types of tobacco	95	96	129	93	98	103	104	102	94	101	97	99
Days in life cycle...	50	50	52	54	54	54	54	61	64	64	71	74

TABLE XVII. *A description of the various types of tobacco used in the life history work on tobacco beetles. All samples were taken May 8, 1928. Both these and the data were furnished by Mr. L. F. Dixon.*

Number	Grade	Dried	Remarks
93	O-E. C '26	9-29-26	Leafy side of South Carolina type. Fairly heavy bodied and compact for the type. Excellent color. Well aged and aromatic.
94	X3-S-C. '26	8-25-26	Very common tips. Some immature green leaves. Thin. Has made all the improvement possible which is very little. Very little aroma.
95	SM-S. C. '27	9-24-27	Good enough for T. Excellent, smooth, well ripened lugs of excellent color. Showed considerable greenish cast at time of sampling. Would produce an aromatic and good smoking tobacco.
96	T-S. C. '26	8-13-26	Average T grade. Typical South Carolina tobacco. Excellent, well aged, good smoking tobacco.
97	T-Dan. '26	10-10-26	Below grade. True to Danville type. Body on light side for the type; color on gray side. Typical Danville aroma.
98	T-Dhm. '26	1-27-27	Very slightly on leafy compact side of the grade. Body slightly heavier than the average. True to type and grade. Well ripened. Well aged and aromatic. Excellent cigarette tobacco.
99	O-Dhm. '26	12-10-26	Leafy compact leaf. Heavy bodied. True to grade and type. Quite aromatic.
100	T-Win. '26	10-25-26	Body lighter than the average. Thin, clean tobacco of color and quality. Slightly below grade. Well aged and aromatic.
101	X3-Dhm. '25	(Dealers)	Heavy bodied, very compact, "slick faced" leaf. Fillery type of tobacco but very old and carrying some aroma.
102	Sp2-Win. '26	12-22-26	Leafy side. Bodied but thin enough to be used in cigarettes. The heavy bodied side of Sp. Carrying considerable tips. Aged well and aromatic.
103	N-Win. '26	11-16-26	True to grade and type. Thin, clear, well grained and ripened tobacco. Aged well and quite aromatic.
104	SM-E.N.C. '26	.....	Thin E. N. C. primings of no body or substance. Undergrade. Poor quality. Very little aroma.
	D-E.N.C.	.....	High grade E. N. C. of fair body, clean well ripened, well aged and aromatic.

The results of these experiments which compare the rates of development of beetles in different kinds of tobacco and under otherwise similar conditions show: (1) that there is a food preference of the beetle for certain types or qualities of tobacco; (2) the preference, as exemplified by the length of life cycle is for the better grades of tobacco; (3) the poorer grades, tend to lengthen the life cycle and are not preferred by the beetles.

ODOR REACTIONS

The reaction of the tobacco beetle to the odors of 21 varieties of tobacco, palm fiber, flax tow, Spanish moss, and yeast cake were tested. Experiments were carried out with 8 types of North Carolina tobacco, one from Virginia, six from South Carolina, and four from Turkey. Tables XVIII and XIX

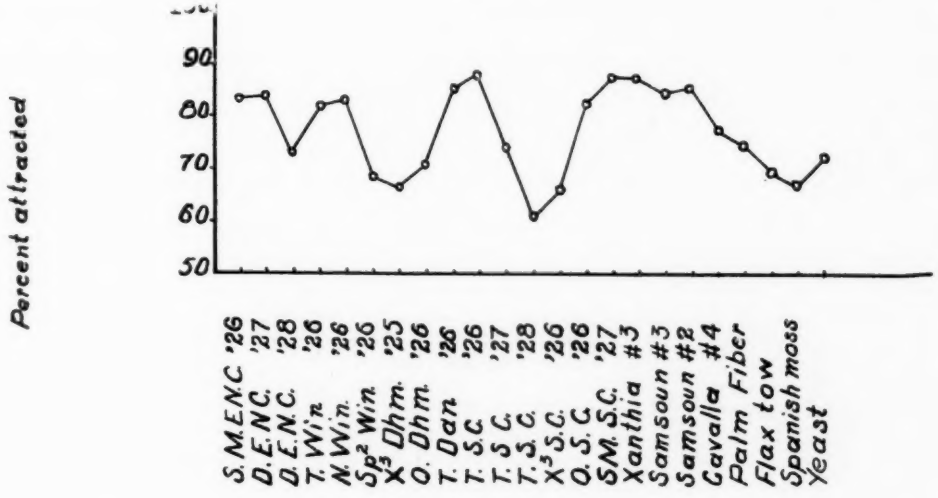

Fig. 15
Showing the reaction of the beetle to various odors from a comparative standpoint

show the types used. A description of each type, except the Turkish will be found in Table XVII.

In running the odor experiments it was found that on cool days the beetles did not leave the reaction tube (Figs. 3, 4) quickly enough. No record is given for experiments at temperatures lower than 25°C., nor higher than 29°C. The substances tested in these experiments were in the original form. Twenty grams of each type of tobacco was placed in a gas washing-bottle and exposed for a period of 24 hours to air which had been drawn through distilled water. Tobacco treated in this fashion gave more uniform results than if the dry leaf were used. The furniture materials and yeast were conditioned

in the same manner, but only 10 grams were used. All of the experiments were conducted under similar conditions.

From Table XVIII it can be seen that the greatest number of positive reactions to tobacco were obtained when certain grades were used. Figure 15 shows that the greatest number of reactions were obtained from South Carolina Tobacco Grade T, crop of 1926. This was 88.57 per cent positive, SM. South Carolina crop of 1927 gave the next highest average, while X^3 Durham crop of 1925 was the lowest of the group.

In this connection another significant factor is brought out, and that is the importance relative to the age of a particular type of tobacco. Figure 15 shows that the highest percentage of positive reactions to a single grade of tobacco was obtained from the oldest. This is shown in Grade D. Eastern North Carolina Tobacco. The 1928 crop induced a positive response of 72.73 per cent, while the 1927 crop gave 84.26 per cent. Another grade showing a

TABLE XVIII. *The reactions of beetles to the odors of various tobaccos, showing the effect of ageing, and also preferences for certain types.*

Each reaction is an average of four experiments, each experiment comprising approximately thirty beetles. The time allowed for individual experiments was thirty minutes. The experiments were made in the following order: 1 and 3, odor on left, control on right; 2 and 4, control on left, and odor on the right. The reaction percentage column is based on the number of beetles that actually reacted positively or negatively to an odor and does not take into consideration those that remained in the container (Figs. 3, 4).

Type material	Grade	Beetle reactions			Temp., °C.	Reaction percentage	
		To odors	To control	Not reacting		To odors	To control
North Carolina Tobacco:							
Eastern—							
1926 crop	SM	86	17	13	28	83.49	16.51
1927 crop	D	91	17	9	26	84.26	15.74
1928 crop	D	80	30	13	26	72.73	27.27
Winston-Salem—							
1926 crop	T	98	21	3	29	82.35	17.65
1926 crop	N	101	20	6	28	83.47	16.53
1926 crop	SP2	75	35	9	28	68.18	31.82
Durham—							
1925 crop	X3	81	41	5	27	66.39	33.61
1926 crop	O	68	28	17	26	70.83	28.17
Virginia:							
Danville—							
1926 crop	T	66	31	14	27	68.04	31.96
South Carolina:							
1926 crop	T	93	12	12	28	88.57	11.43
1927 crop	T	107	36	2	25	74.82	25.18
1928 crop	T	68	44	8	26	60.71	39.29
1926 crop	X3	72	37	4	28	66.06	33.94
1926 crop	O	82	17	16	28	82.83	17.17
1927 crop	SM	80	11	28	27	87.91	12.09

similar difference is T. South Carolina. From the 1928 crop positive reactions amounting to 60.71 per cent were obtained, there was an increase to 74.82 per cent with the 1927 crop, and the 1926 crop gave 88.57 per cent. Mr. L. F. Dixon states that the particular grade of tobacco for two successive years might not be identical, because of seasonal differences. The actual grading of the tobacco will have a slight variation because it is not all graded by one person, but the results here are large enough to admit of slight variations and still remain significant. In the case of Turkish Tobacco high percentages of positive reactions were obtained, the highest being for Xanthia No. 3 (87.97%), while the lowest was for Cavalla No. 4 (77.69%). Of the furniture materials tested the order of preference shown was palm fiber, flax tow, and Spanish moss. Magic yeast gave a reaction of 72.07 per cent.

TABLE XIX. *The reactions of beetles to the odors of five grades of Turkish tobacco, and several other food substances.*

Each reaction is an average of four experiments, each of which included approximately thirty beetles. The time allowed for individual experiments was thirty minutes. The experiments were in the following order: Nos. 1 and 3, odor on left, control on right; Nos. 2 and 4, control on left, and odor on the right. The "Reaction per cent" column is based on the number of beetles actually reacting to or against the odor and does not take into consideration those that remain in the reacting bottle.

Type of material	Beetle reactions			Temp., °C.	Reaction percentage		Amount material, grams
	To odor	To control	Not reacting		To odors	To control	
Turkish tobacco:							
Xanthia No. 3	95	13	20	27	87.97	12.03	20
Samsoun No. 3	88	16	15	27	84.61	15.39	20
Smyrna No. 2	115	19	8	28	85.82	14.18	20
Smyrna No. 4	94	27	14	26	77.69	22.31	20
Cavalla No. 4	81	29	14	25	73.64	26.36	20
Palm fiber	79	27	9	27	74.53	25.47	10
Flax tow	69	30	20	27	69.69	30.31	10
Spanish moss	80	40	9	27	66.67	33.33	10
Yeast sized	80	31	16	28	72.07	27.93	10

Table XIX shows the results obtained from experiments with tobacco, furniture materials, and yeast. These indicate: (1) that the tobacco beetle manifests preferences in its reactions to odors; (2) the aroma from what a man esteems as the better grades of tobacco is more acceptable to the beetle than that from the poorer types; (3) aging of the average and better classes of tobacco apparently improves the aroma so that the beetle shows a percentage of positive reactions in proportion to "age"; (4) aging of the heavy-bodied, fillery type, or poorer grades, improves the aroma but little, so far as the beetle is concerned; (5) a preference is shown by the beetle, for Turkish tobacco; (6) furniture materials and yeast are comparable to unaged tobaccos, as judged by olfactory responses.

TEMPERATURE

The value of high temperatures as a means of controlling beetles was investigated. It was found in the life-history series that a constant temperature of only 40°C. (105°F.) was fatal to all stages of the beetle. Previous to this experiment an exposure to 44°C. for a period of 10 days killed all stages of the insect. Table No. XX shows a summary of the results obtained. The temperatures and humidities indicated were selected because they are similar to those encountered in the manufacturing process of many products of tobacco. It was noticed that the eggs were the most difficult stage to destroy by means of heat. In no instance, however, did a beetle reach maturity that had hatched from an egg exposed to the temperatures indicated. These experiments show: (1) that high temperatures are an effective way of destroying the tobacco beetle; (2) tobacco containing beetles must be exposed to a high temperature sufficiently long for the heat to penetrate through it.

TABLE XX. *The effect of high temperature on various stages of the tobacco beetle.*

Temperature °C.	Humidity percent	Length of exposure	Number of each stage				Percentage killed
			Eggs	Larvæ	Pupæ	Adults	
44	62	10 days	20	20	12	20	100
55	Saturated	3 min.	12	12	12	12	None
77	Saturated	6 min.	12	12	12	12	100*
84	Saturated	20 min.	12	12	12	12	100
35–95	Saturated	50 min.	12	12	12	12	100
42–100	Saturated	20 min.	12	12	12	12	100

*Two eggs later hatched, but the larvae did not mature.

LOW TEMPERATURES AS A MEANS OF CONTROLLING THE TOBACCO BEETLE

Experiments were conducted to find out what effect low temperatures exerted on various stages of the tobacco beetle. First a constant temperature of 22°C. (70°F.) was used. Eggs, larvae, and pupae were the stages employed. It is evident that the pupae were hardier at this temperature than any of the other stages. Nearly one-half (47.50%) of them emerged as adults. Eggs were also quite hardy but larvae suffered considerable mortality and only 19.17 per cent of them pupated. Eggs were kept in cold storage (2°C.) for a period of 20 days and then incubated at 32°C. Out of a total of 105 eggs treated in this manner 45 hatched, while 60 failed to hatch.

These experiments show: (1) the pupa is the most resistant stage of the tobacco beetle, when exposed to a constant temperature of 22°C.; (2) temperatures above 2°C. result in a high mortality rate, but do not sterilize tobacco; (3) the eggs of the beetle can withstand a constant temperature of 2°C. for a period of 20 days, and still approximately fifty per cent will hatch; (4) after an exposure of 20 days to 2°C. the incubation period of the eggs is

of about the same length as those which are incubated immediately upon being laid (Table III).

TEMPERATURE OF TOBACCO IN STORAGE

The hogshead temperature of tobacco in storage fluctuates as the seasonal temperature varies. Daily fluctuations of temperature are not apparent in

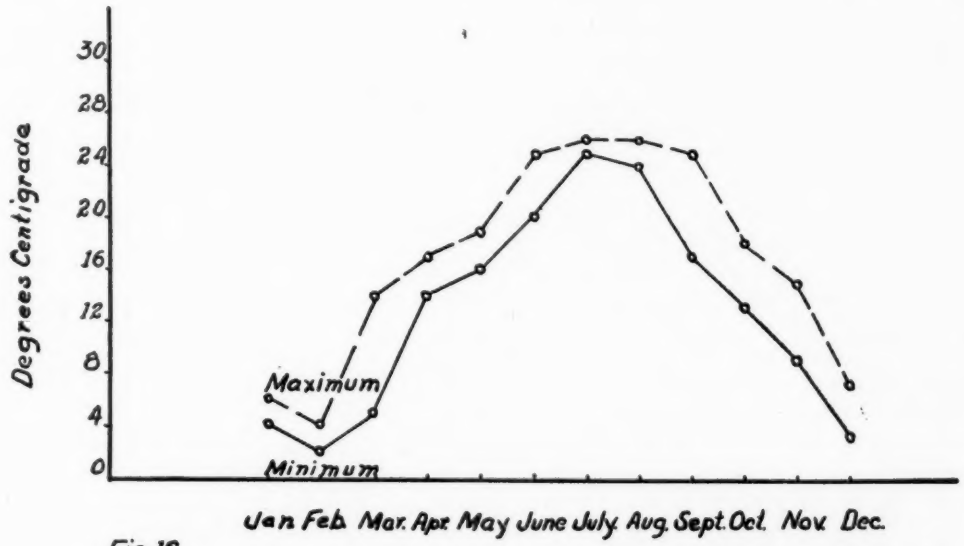

Fig. 16
Showing the monthly maximum and minimum hogshead temperatures in tobacco during 1929 at Durham, N.C.

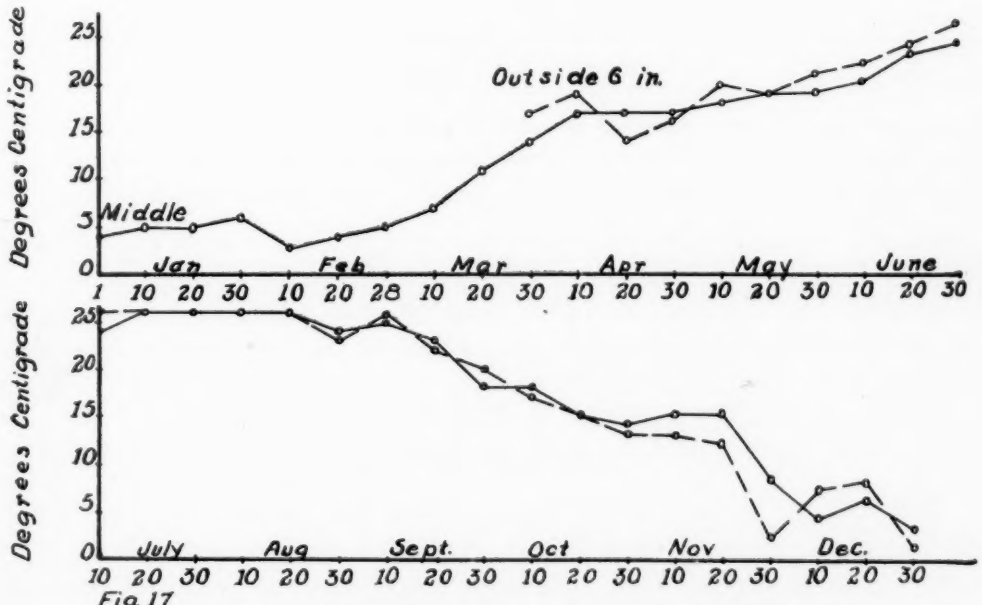

Fig. 17
Showing the temperature of tobacco in hogsheads taken at Durham, N.C. during the year 1929

the hogshead. There is present a more or less constant condition. Table XXI and Fig. 16 show the monthly maximum and minimum hogshead temperatures. These figures were furnished by Mr. L. F. Dixon, who made daily records of hogshead temperatures throughout the year 1929. Table XXII and Fig. 17, from date furnished by Mr. Dixon, show temperatures taken from the middle of the hogshead and in the outer 6 inches of tobacco. The latter figures are particularly valuable since beetles do not usually penetrate more than 6 inches into a hogshead. Figures are given for three dates in each month. These tables and graphs are presented to show the actual temperatures present in hogsheads of tobacco, while in storage, for a period of an entire year.

TABLE XXI. *The monthly maximum and minimum hogshead temperatures in degrees centigrade in tobacco at Durham, N. C. during the year 1929. Data for this table furnished by Mr. L. F. Dixon.*

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum	6	4	14	17	19	25	26	26	25	18	15	7
Minimum	4	2	5	14	16	20	25	24	17	13	9	3

TABLE XXII. *The temperature in degrees centigrade of tobacco in hogsheads taken at Durham, N. C., during the year 1929. All data in this table was furnished by Mr. L. F. Dixon.*

Months	January				February		March				April			May			June		
Date	1	10	20	30	10	20	1	10	20	30	10	20	30	10	20	30	10	20	30
Outside 6 inches										17	19	14	16	20	19	21	22	24	26
Middle of Hogshead	4	5	5	6	3	4	5	7	11	14	17	17	17	18	19	19	20	23	24

Months	July			August			September			October			November			December		
Date	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	26
Outside 6 inches	26	26	26	26	26	23	26	22	20	17	15	13	13	12	2	7	8	1
Middle of hogshead	24	26	26	26	26	24	25	23	18	18	15	14	15	15	8	4	6	3

FUMIGANTS

Hydrocyanic-Acid Gas

In the experiments on hydrocyanic-acid gas, the gas was generated on the outside of the fumigation tank (Fig. 4) by the use of sodium cyanide and acid in a 500 c.c. Erlenmeyer flask. The flask was attached to the top inlet of the tank, while the gas was being administered. Table XXIII and Fig. 18 show the results obtained when hydrocyanic-acid gas was used as a fumigant. Nine

experiments were conducted, all for 24 hours. The concentrations used in the experiments varied from 10 to 40 ounces of HCN per 1000 cu. ft.

When a concentration of 10 ounces of HCN per 1000 cu. ft. was used, only 10.3 per cent of the beetles were killed. This was true when two presses of tobacco were used. The concentration in the tank was sufficiently strong to kill only 48.87 per cent of the control beetles which were not buried in the tobacco. Numerous citations in the literature give this as a fatal dose for the tobacco beetle. To discover whether or not the tobacco present in the presses had anything to do with the low per cent killed, another experiment was carried out in which the tobacco was left out; all other conditions being the same. 100 per cent of the beetles died. This demonstrated conclusively that the tobacco interfered in some way with the lethal effect of the gas.

TABLE XXIII. *Hydrocyanic acid gas used as a fumigant for 24 hours.*

Exp. No.	NaCN in 8.58 cu. ft. grams	HCN in 1000 cu. ft., ounces	Beetles killed				Temperature	
			Number		Percent		Max.	Min.
			Press	Control	Press	Control	°C.	°C.
1	4.48	10	8	3	10.30	48.87	28	24
2*	4.48	10	78	7	100.00	100.00	31	27
3	6.72	15	11	?	14.10	?	30	25
4	8.96	20	14	4	17.90	57.14	30	26
5**	8.96	20	28	7	35.90	100.00	28	24
6	11.20	25	19	7	24.40	100.00	30	27
7	13.44	30	25	7	32.00	100.00	30	28
8	15.68	35	30	7	38.50	100.00	30	27
9	17.82	40	40	7	47.40	100.00	31	28

*No tobacco was used in this experiment.
**Only one press, or one-half of the regular amount of tobacco was used.

With increasing amounts of HCN there was greater mortality among the beetles (Fig. 18). With 20 ounces of HCN per 1000 cu. ft., a test was made of the effect of the presence of the tobacco on the gases present in the tank. An experiment was carried out using the same concentration of gas but half the amount of tobacco and the results were significant. The increase efficiency of the gas when only one press was used was more than doubled. The fumigation tank in which these experiments were conducted contained a total of 8.58 cu. ft. of space. A single press of tobacco occupied an area of 2197 cu. in. or 1.27 cu. ft., and the two presses together, 2.54 cu. ft. With HCN at the concentration used, 2.54 cu. ft. of tobacco reduced the lethal effect of the gas to only 17.9 per cent. When one press, or 1.27 cu. ft., of tobacco was present the lethal effect of the gas was raised 18 per cent to a total of 35.9 per cent of the beetles killed.

It is to be noted that all the beetles in the control were not killed until the concentration reached 25 ounces of HCN per 1000 cu. ft., which shows that all weaker concentration can not be depended on to penetrate into the tobacco

and kill sheltered beetles. At this concentration the gas penetrated well to a depth of about half an inch. When 30 oz. of HCN per 1000 cu. ft., were present, penetration reached 1.5 inches. The penetration increased to 2.5 inches with 35 ounces and to 3.5 inches with 40 ounces of HCN per 1000 cu. ft.

The results of experiments with hydrocyanic-acid gas as a fumigant, and with exposure to the gas for 24 hours, show: (1) that in the total absence of tobacco a concentration of 10 ounces of HCN per 1000 cu. ft. kills 100 per cent of the beetles; (2) the lethal effect of the gas is reduced in proportion to the amount of tobacco present; (3) an increase amount of HCN results in a proportional increase of the number of beetles killed; (4) if all beetles buried in tobacco to a depth of 3.5 inches are to be killed, at least 40 ounces of HCN per 1000 cu. ft. should be used, when the amount of tobacco present occupies as much as 30 per cent of the space to be fumigated.

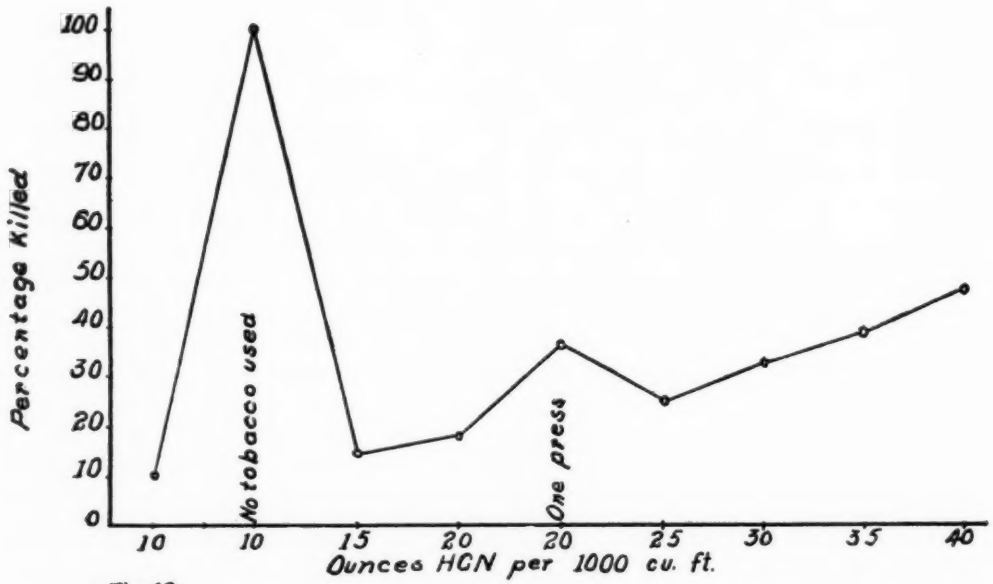

Fig. 18
Showing the effect of hydrocyanic gas on the tobacco beetle

CARBON DISULPHIDE

In experimenting with carbon disulphide the gas was generated on the outside of the container, as for HCN. Liquid carbon disulphide was weighed out and placed in an Erlenmeyer flask. This material being heavier than air was run into the tank through the bottom inlet. The actual method of getting the gas into the fumigation compartment consisted of attaching the flask containing the liquid carbon disulphide to the bottom inlet and then slowly heating the flask. This caused the liquid to be driven into the compartment in a gaseous form. The length of time necessary to get the gas into the container

varied with the amount of the liquid but not over 20 minutes was required for the heaviest charge. Table XXIV and Fig. 19 show the concentrations used and the results obtained. Eight experiments were performed all for 24 hours. The smallest concentration was 0.5 pound per 1000 cu. ft. and the largest 7 pounds for the same volume.

10.30 per cent of the beetles were killed when a concentration of 0.5 pound per 1000 cu. ft. was used. A rapid increase in deaths occurred when the concentration of the fumigant increased to 0.75 lb. per 1000 cu. ft. The results show a 100 per cent mortality in the control, and 88 per cent for beetles in the presses. Only a few beetles escaped near the center of the tobacco at this concentration.

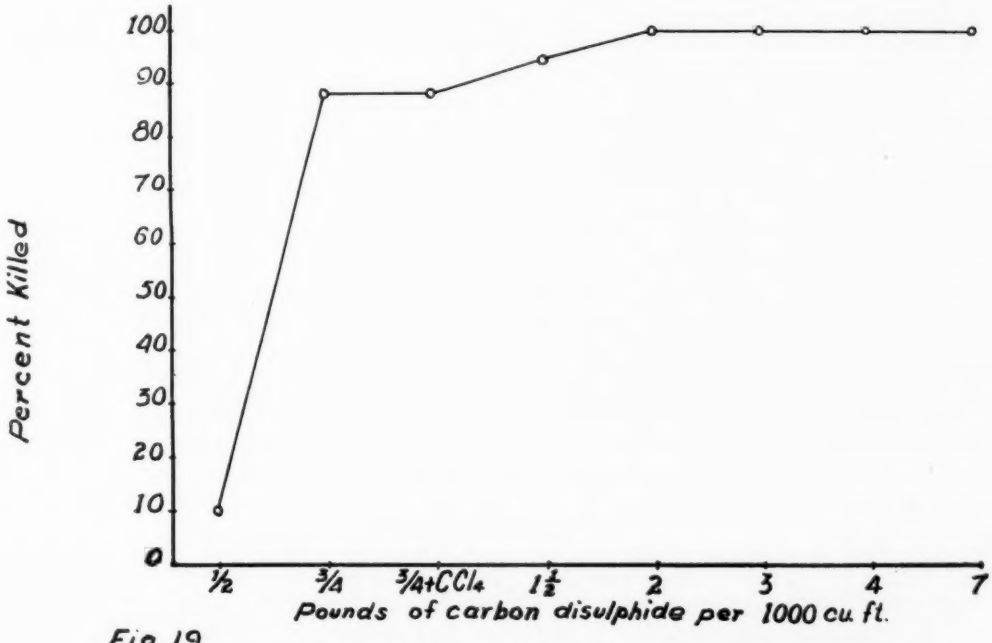

Fig. 19
The effects of carbon disulphide on the beetle

Carbon disulphide vapor is highly inflammable and explosive when mixed with air in certain proportions. It was desired to note what effect carbon tetrachloride would exert on the per cent of beetles killed by carbon disulphide. This information was desired because carbon tetrachloride is sometimes mixed with carbon disulphide to reduce the fire hazard attendant on its use. An experiment was therefore performed in which a mixture of the two substances was used. The mixture consisted of 3 parts of carbon disulphide and one part of carbon tetrachloride by volume. The amount of carbon disulphide effective was 0.75 lb. per 1000 cu ft. Table XXIV shows that the same results were obtained from this experiment as when no carbon tetrachloride was used. This demonstrated that the amount of carbon tetrachloride used did not appreciably increase or decrease the lethal effect of the carbon disulphide gas.

1.5 pounds of carbon disulphide per 1000 cu. ft. killed nearly all the beetles exposed to it in tobacco (94.9%) but not until 2 pounds of the fumigant per 1000 cu. ft. were employed did the lethal effect become 100 per cent. All higher concentrations killed all the beetles.

The results of the experiments with carbon disulphide on the basis of exposures of 24 hours show: (1) that carbon disulphide is an effective gas against the tobacco beetles when they are enclosed in masses of tobacco; (2) that the minimum lethal dose for a beetle embedded 6.5 inches in tobacco is 2 pounds of the liquid to 1000 cu. ft. of space; (3) and that carbon tetrachloride when used with carbon disulphide does not reduce or increase the effect of the latter.

TABLE XXIV. *Effects of carbon disulphide as a fumigant when applied for 24 hours.*

Exp. No.	Amount of carbon disulphide per 1000 cu. ft. in pounds	Beetles killed					
		Number killed		Percent killed		Temperature	
		In press	In control	In press	In control	Max. °C.	Min. °C
1	0.50	8	2	10.30	28.57	29	26
2	0.75	69	7	88.50	100.00	32	28
3*	0.75	69	7	88.50	100.00	31	26
4	1.50	74	7	94.90	100.00	28	24
5	2.00	78	7	100.00	100.00	27	24
6	3.00	78	7	100.00	100.00	33	27
7	4.00	78	7	100.00	100.00	29	24
8	7.00	78	7	100.00	100.00	33	28

*One part carbon tetrachloride to three parts of carbon disulphide was used.

ETHYLENE OXIDE

Three experiments were conducted to determine the comparative value of ethylene oxide as a fumigant. This gas was administered through the bottom of the fumigation tank (Fig. 4). The liquid ethylene oxide was weighed out and placed in a flask. The boiling point of this liquid is 10.5°C., so that the liquid is converted into a gas at room temperature. Table XXV and Fig. 20 show the results obtained. A penetration of 6.5 inches accompanied by a mortality of 100 per cent occurred with a charge of 1.5 pounds of the liquid ethylene oxide per 1000 cu. ft. A one pound charge of the material killed 78.2 per cent in the presses and 100 per cent in the control.

The results from experiments when ethylene oxide is the fumigant used and when exposures are for 24 hours show: (1) that ethylene oxide is an effective gas when used against the tobacco beetle; (2) the minimum lethal dose for a penetration of 6.5 inches through tobacco is 1.5 pounds of the liquid for each 1000 cu. ft. of space.

Two experiments were carried out in which a mixture of ethylene dichloride and carbon tetrachloride were used. These substances were mixed in the

proportion of three parts of ethylene dichloride to one part of carbon tetrachloride by volume. The gas was run into the bottom of the fumigation tank (Fig. 4) by means of heat. Table XXV and Fig. 20 show the results obtained. When 3 parts of ethylene dichloride and one part of carbon tetrachloride are mixed and used as the fumigant, an exposure of 24 hours gives results which indicates: (1) that such a mixture is not required to satisfactorily kill tobacco beetles; (2) a charge of 4 pounds per 1000 cu. ft. of air will kill only 19.2 per cent; (3) satisfactory results can be obtained only when a volume much larger than 10 pounds per 1000 cu. ft. is used.

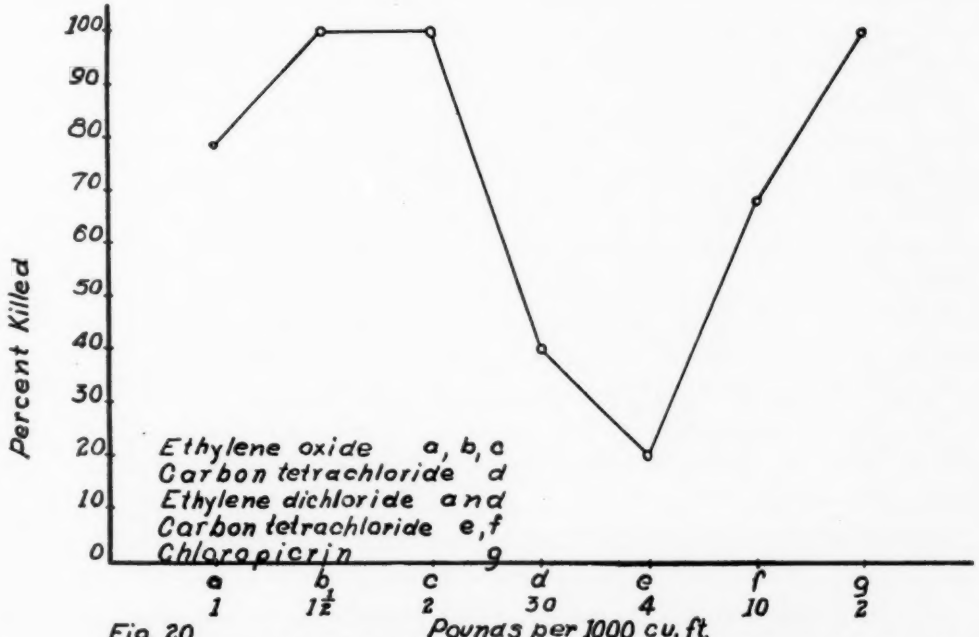

Fig. 20
Showing effect of ethylene oxide, carbon tetrachloride, ethylene dichloride and carbon tetrachloride, chloropicrin on the beetle

One experiment was performed in which carbon tetrachloride alone was used. This was to see if this substance would kill beetles without the aid of any other gas. The gas was administered into the bottom inlet of the fumigation tanks (Fig. 4). Heat was necessary to convert the liquid into a gas and force it over into the fumigation chamber. Table XXV and Fig. 20 show the results obtained. A charge of 30 pounds per 1000 cu. ft. killed only 39.7 per cent of the tobacco beetles. The results from this experiment based on exposures of 24 hours, show: (1) that this substance is not an efficient gas for use in combating tobacco beetles; (2) large amounts of the gas are necessary to have any effect on the beetle; (3) a charge of 30 pounds per 1000 cu. ft. does not kill 50 per cent of the beetles while buried in tobacco.

Only one experiment was performed with chloropicrin as a fumigant.

Liquid chloropicrin (37.82 g.) was weighed out and placed in a flask. This was on the basis of 2 pounds per 1000 cu. ft. The material was converted into a gas by means of heat and administered through the bottom inlet to the fumigation tank (Fig. 4). Table XXV and Fig. 20 show the results obtained as a result of exposures of 24 hours: (1) chloropicrin is highly toxic to the tobacco beetle; (2) a charge of 2 pounds of chloropicrin per 1000 cu. ft. is effective as a control.

TABLE XXV. *Fumigations with ethylene oxide, carbon tetrachloride, carbon tetrachloride and ethylene dichloride and chloropicrin. All experiments were run for a period of 24 hours.*

Fumigant	Concentration		Beetles killed				Temperature	
	8.58 cu. ft. grams	1000 cu. ft. pounds	Number killed		Percent killed		Max. °C.	Min. °C.
			In press	In control	In press	In control		
Ethylene oxide...	18.91	1.0	61	7	78.2	100.00	33	27
	28.37	1.5	78	7	100.0	100.00	32	25
	37.82	2.0	78	7	100.0	100.00	35	28
Carbon tetrachloride...	567.30	30.0	31	1	39.7	14.28	32	27
Ethylene dichloride (3).. and Carbon tetrachloride (1)....	75.64	4.0	15	1	19.2	14.28	30	28
	139.10	10.0	53	5	67.9	71.43	30	28
Chloropicrin.....	37.82	2.0	78	7	100.0	100.00	31	26

DISCUSSION

The experimental results recorded in this paper show that the following factors influence the length of the life cycle of the tobacco beetle: (1) food, (2) temperature, and (3) humidity.

Food has been recognized as a determining factor in the length of the life cycles of insects for some time. Chapman (1918) states that a comparison of the rates of development of beetles in various foods, under uniform conditions, has shown that the life cycle may be slightly longer in some foods than in others. This condition is also well demonstrated by the tobacco beetle. The writer's experiments showed that the length of the life cycle was 20 days longer in tobacco than in yeast under the same conditions. In considering these facts it is well to remember that there are individual variations in the period of egg incubation and length of the larval stage within the same type of food. The writer's experiments, however, involved such large numbers of beetles as to compensate for individual differences and still give a dependable result.

In (1926) Richardson showed that Vitamin B is necessary for the normal

growth of certain insects. Since yeast is one of the richest known sources of this vitamin, it is quite probable that this explains why the yeast affords a better food for the tobacco beetle than the tobacco. Another factor in this connection is that the tobacco beetle feeds on its food substance only in the larval stage. Loeb and Northrop (1917), while working with *Drosophila melanogaster,* found that the larvae required yeast, but that the adult could live on other materials. Since the adult of the tobacco beetle does not require food, it follows that the optimum food of the larva of this insect, is all that requires consideration.

In regard to temperature, the optimum for the tobacco beetle was found to be near 32°C., since the shortest life cycle occurred there. This was true when beetles were reared in yeast or in any of the types of tobacco used. The experiments show that the tobacco beetle can exist at temperatures between 2°C. and 36°C. Eggs were the only stages tested at temperatures from 2°C. to 22°C. These showed a high mortality rate, but it is of interest that an exposure of 20 days to a constant temperature of 2°C. caused a mortality of only 60 per cent. The chief effect of unfavorable temperatures on this insect is to prolong the life cycle. The length of the life cycle was increased from 28 days at the optimum to a total of 55 days at 24°C.

The lower limit at which a constant temperature will support beetle life was not determined, since none of the experiments were run so low as to cause the death of all beetles. The higher limits were found to exist somewhere between 36°C. and 40°C. Glenn (1922) assumed that the rate of development of the Codling moth increased regularly up to an optimum temperature and then decreased at the same rate. For example, if the maximum rate was at 90°F. he considered 100°F. equivalent to 80°F. etc. The results shown in this paper differ from those obtained by Glenn, in that the values above the optimum fall more rapidly than those below. Shelford (1927) recognized a lag period in the development of insects and sums up the situation by stating that "the evidence for the deviation of the developmental velocity from a straight line at low and high temperatures is strong, and there is no reason why procedure should not be based upon the facts."

The activity of the beetle is limited by temperature. Cool days cause the insect to become so sluggish as to remain in open glass vials (page 372), while on hot days they are difficult to confine. Such degrees of activity were quite noticeable in the constant temperature controls. Andrews (1927) measured the speed at which ants travel and showed that the rates fall off with lower temperatures. He also suggests that the small size of the north as compared with the south part of bilateral ant mounds may be due in part to direct effects of sunshine in speeding up the work of the ants upon the warmer surfaces of the mound. This leads us to a more serious consideration of the optimum for the insect.

A temperature of 32°C. is not optimum for the bodies of warm blooded animals nor is it optimum for vertebrate enzymotic activity. Pearse and Hall (1928) state that enzymes are effective only through a very well defined range of temperatures, and that their maximum rate of reaction takes place at optimum temperatures which are very near those of the bodies of warm blooded animals. In this connection it might be well to note that the body temperature of man is approximately 16°C. higher than the optimum room temperature. Pearse and Hall (1928) state further that the body temperatures of cold blooded animals may differ as much as several degrees from that of the environment, but usually such temperatures resemble each other very closely. Thirty-two degrees C. is then the optimum for the air in which the insect lives and is not necessarily the optimum for the body. That other factors are involved is well shown in that a low humidity even at 32°C. lengthens the life cycle and thus destroys the optimum condition. It would seem probable that 32°C. represents the optimum temperature medium for all of the factors involved in the metabolism of the beetle.

The effect of humidity on the length of the beetle life cycle should be considered in connection with temperature. It is apparent that the moisture to which the insect is exposed vitally affects the organism. This is shown by the fact that humidities lower than 45 per cent were always fatal, and the same is true for humidities above 90 per cent. At every temperature tested experimentally by the writer, 75 per cent was the optimum humidity. From a low point at 45 per cent, the humidity curve rises to the optimum and then declines. This means that humidity is an actual factor in the metabolic activity of the beetle, which is conclusively shown when a number of experiments were started under identical conditions, except for humidity, and all came to maturity in a regular order which was directly related to the percentage of moisture present.

The lower humidities were apparently fatal because of desiccation. All of the other factors were controlled except the variation in humidity. These results apparently support the experiments of Hall (1922). While working on the vital limits of desiccation he found that the meal worm, *Tenebrio molitor* Linn., could be dried until it lost 52.6 per cent of its body weight and live, but a greater degree of desiccation resulted in death. He showed further that the meal worm endured desiccation for a considerable length of time. The vital limit of desiccation in the tobacco beetle occurs between 45 and 30 per cent humidity, since the insect lives in the former and not in the latter. Humidities above 90 per cent are unfavorable for the insect, because of the growth of mold. It has been often observed in the tobacco storage warehouses that when the tobacco molded, all beetles were killed.

The viability of eggs shows the toleration of tobacco beetles for various humidities. In the writer's experiments as the humidity increased the num-

ber of viable eggs decreased from 100 per cent to only 13 per cent at 75 per cent humidity. At 45 per cent humidity, 39 per cent of the beetles failed to emerge; at 60 per cent humidity, 32 per cent; and 90 per cent humidity, 26 per cent. These figures mean that at 32°C. and 75 per cent humidity, 38 adults matured from the average of 43.57 eggs laid by a single female beetle. The number maturing at 90 per cent humidity was 32; at 60 per cent 30; and at 45 per cent, 27. These figures are based on results when yeast was used as food and the temperature was 32°C. The results show that a low humidity and a low temperature tend to reduce the number of beetles which mature.

The number of tobacco beetle generations in a given time is dependent upon the seasonal temperature and humidity. Runner (1917) states that "in the latitude of Virginia and Tennessee there seems to be a period of greater abundance of the adults coinciding with the first warm weather in June, and again in August and early September." Two swarms of beetles each summer are also characteristic in the tobacco warehouses in North Carolina.

Numerous hogsheads of tobacco were examined in the spring of 1929 and the beetles were usually found in the larval stage. The temperature in the warehouses at the time of these observations had not reached 24°C. The indications are that the beetle winters in the larval stage and when the temperature becomes favorable in the spring, pupates and emerges as an adult. A situation of this kind would account for the emergence of great numbers of adults during the month of June. Prevailing temperatures would make it manifestly impossible for these insects to have developed from eggs laid during the spring.

In Table XXII figures are given which show hogshead temperatures for each month of the year. The outside 6 inches are the most important from the standpoint of the beetles because rarely does one find infestation by these insects more than 6 inches deep. It is of interest to consider these figures in connection with life history data. The tobacco temperatures average 24°C. for June, 26°C. for July, 25°C. for August and 23°C. for September. This gives an average of 24°C. for the four months of the summer when the beetle infestation is the heaviest. The table shows that it is June the 20th before the temperature attains 24°C. This is approximately the time of the first swarm. Mr. L. F. Dixon states that the humidity normal in hogsheads ranges from 50 to 55 per cent. The writer's work on the length of the life cycle of the beetle under controlled conditions shows that at a temperature of 24°C. and a humidity of 45 per cent a complete life cycle is passed through in about 83 days. A humidity of 60 per cent at this temperature reduces the time required to 79 days. By analogy a period of 81 days may be assumed for a humidity of 52 per cent. A period of 81 days from the 20th of June, would place the time for the second swarm on the 9th of September, which is approximately the correct date for this latitude. It is quite apparent that a

modification of either temperature or humidity would cause a corresponding change in beetle swarms. With a rise in temperature, three and even more swarms might occur. A very early spring followed by a late fall might effect the hogshead temperature sufficiently to bring about such a condition.

The experiments described in this paper show that oviposition commences within a 24 hour period following copulation. Under normal conditions copulation occurs on the day of the emergence. The length of the oviposition period, about 8 days, is approximately the same, irrespective of whether it occurs immediately after emergence or is delayed for a longer or shorter time up to 10 days.

Records of the number of eggs laid by single individuals were obtained under room conditions and also under controlled conditions. The average number of eggs obtained under controlled conditions was always greater than under room conditions. Controlled conditions resulted in an average of 43.57 eggs per female beetle and under room conditions the average was only 24 eggs per female. Runner (1917) gives the average of from 25 to 30 eggs per female. These figures correspond to the writer's results under room conditions. The reason why the number of eggs obtained under room conditions gives too low an average is due primarily to the age of the female. When beetles are caught in the tobacco storages, there is no way of determining their correct age. In many cases the females may have already laid a considerable number of eggs. This is true even though the insects are caught copulating, because the experiments show that beetles may mate more than once. Other factors such as humidity and temperature are also concerned. During the writer's observations the beetles would never lay eggs on totally dry substances. It is quite probable that tobacco dries out under room conditions to such an extent that the insects would not oviposit. The only dependable average must be obtained from beetles which are caught immediately upon emerging and kept under controlled conditions. There are a number of factors influencing oviposition in the tobacco beetle. The most important of which are: (1) presence of suitable materials; (2) fertilization; (3) physical form of materials; (4) odors.

The writer's oviposition experiment shows that in no case will the beetle lay eggs in the absence of materials upon which to lay. It is not essential for the material to consist of tobacco as other substances, such as yeast, will induce the laying of eggs. The essential thing is that some type of substance which more or less resembles a dried tobacco leaf must be present. Many beetles were kept in empty glass jars during their whole lives without ever laying. Jones (1913) states that apparently unfertilized females will not lay eggs. The experiments reported here confirm this observation. In numerous instances solitary females were kept for whole life periods without copulation, and laid no eggs. Physical form of materials alone was found to exert an

influence on oviposition. A careful survey of the literature pertaining to insects does not reveal the fact that any other investigator has found physical form to be a sufficient stimulus to cause oviposition. Chapman (1918) working on the flour beetle, showed that this insect would invade a coarse medium of sawdust, but he does not state that the beetle oviposited while present in that material. He sums up the situation in his paper by stating that the "coarseness of the flour or cereal may be the dominant factor concerned in invasion by insects."

To exclude all factors such as nutrition and odors, certain insoluble, odorless inorganic substances were used for tests. The list of materials in which eggs were laid includes: iron-filings, wool, glass wool, potassium dichromate, sawdust, sand, and crushed glass. The idea was not to see how many eggs would be laid on each substance, but rather to see which would induce oviposition.

The factor in connection with these experiments, which offers the most conclusive proof that the size of particle concerned was the controlling stimulus in oviposition was that of sizing the materials. Sand, crushed glass, and sawdust were carefully separated out into sizes. The sizes ran from very coarse materials (00000) to the smaller particles. In all instances eggs were found throughout the smaller particles but they were not found in the coarser materials. If size of particle did not act as a physical stimulus to cause oviposition, one would expect to find eggs throughout the series. The fact that similar results were obtained in all three materials makes it seem evident that physical form and size of particles are the controlling factors in these experiments. The beetles respond to the finer materials between which they can penetrate very readily while the more coarsely granular material does not offer the same stimulus. Perhaps the ease of secluding the eggs in the finer sized particles helps to render such substances acceptable as a place for oviposition. This is true when the beetle oviposits on the tobacco leaf. The eggs are found well concealed in the creases and folds of the leaf. The reason why physical form is such an efficient factor in bringing about oviposition is not clear. Probably the mechanical stimuli produced by very fine materials such as sand, glass, etc., result in the same sensation to the beetle as those obtained when the beetles are present on the surface of a tobacco leaf. Over the body surface of the beetle there is a fine layer of hair and the surface of a tobacco leaf is also covered with hair. It is conceivable that the beetles recognize the presence of tobacco from physical contact and react in the same manner—that is oviposit—on any substance which is small enough to give the same sensation as the hairs on the tobacco leaf.

Odors were tried as a stimuli to produce oviposition. Hot water extracts of the tobacco leaf and varying percentages of nicotine caused the beetle to lay a few eggs. In no instance, however, did the odors tried approach phys-

ical form in effectiveness as a stimulus for oviposition. From these results it appears that physical form is a stronger stimulus for oviposition than odors.

Food preferences of the tobacco beetle are indicated by the following: (1) length of life cycle in different sizes of tobacco particles; (2) length of life cycle in different types of food; (3) reaction of the adults to odors from the tobacco leaf. The basis for the view that the beetle shows a preference for tobacco particles of certain sizes is brought out when the length of life cycle is increased from 51 days in particles passed through a number 00000 sieve to a length of 70 days in particles passing through a number 7 sieve. If the tobacco were of different grades, or even of different ages it might account for such a difference. In the case in question, however, all factors other than size were identical. In fact the materials were all ground together at one time and were obtained from the same kind of tobacco. It is interesting to note in this connection that the normal length of the beetle life cycle for this type of tobacco is 52 days (Table XVI). These results were obtained from tobacco ground but not sieved. It appears that the life cycle is of normal length in the coarser particles, while those of smaller size cause it to be lengthened. Probably the smaller sized particles of tobacco enter the spiracles of the beetle, thereby affecting metabolic activity. Inability to culture beetles in tobacco as fine as snuff makes it seem reasonable to assume that such small particles have an insecticidal effect.

When the tobacco beetle is reared in different types of tobacco, its life cycle is shorter in some than in others. The shortest life cycle is found in that tobacco known to experts and users as the "better type." The type of tobacco described as "heavy bodied," yields the longest life cycle. In general the heavy bodied type of tobacco contains more nicotine (Mr. L. F. Dixon) than the better types. Using the length of life cycle as a criterion the better types are to be preferred by beetles. Preference for certain types of tobacco is conclusively shown by the olfactory responses of the beetles. In experiments concerning these the proof lies in the fact that the adult beetles choose the type of odor preferred and goes into that environment. The facts show a differential percentage of response to particular aromas. The aroma of tobacco is improved, and at any rate causes a larger percentage of positive reactions by a process of aging. Barrows (1907) has shown that Drosophila does not find its food by sight, but by smell. The work of McIndoo (1927) shows that various plants attract insects by emitting odors. The results presented in this paper apparently support the work of these two investigators. In order to be sure that no other sense aided the olfactory responses an apparatus (Fig. 3) was used which practically eliminated or controlled all the stimuli except odors. By the use of this apparatus it is well shown that beetles prefer the "better types" of tobacco by choosing the aroma from such types. It may be that the high nicotine content of the

heavier bodied types of tobacco renders them objectionable. It has been shown (p. 46) that the fumes from several drops of a nicotine solution stronger than 3 per cent results in death to the beetles, even though they are not closely confined. This would seem to indicate that nicotine exerts an insecticidal effect even on the tobacco beetle and hence an aroma too heavily laden with nicotine would be rejected for one of a lower concentration.

Gases have been used for a considerable period to rid tobacco of beetles. The two most important have been hydrocyanic acid and carbon disulphide. Hydrocyanic acid gas is fatal to the insect provided the concentration is strong enough. It is quite probable that many unsatisfactory results obtained with this gas were due to too weak a dosage. Runner (1917) states that 10 ounces of potassium cyanide per 1000 cu. ft. will kill all beetles within an air tight compartment. The experiments reported in this paper agree with those of Runner to a certain extent. At such a concentration all beetles were killed unless they were enclosed in tobacco. If tobacco is present the mortality among beetles will depend on the amount of tobacco and the concentration of the gas. This was well shown when an experiment was conducted in which gas from 20 ounces of sodium cyanide per 1000 cu. ft. was used and only 17.9 per cent of the beetles were killed. The lethal effect of gas at this concentration was raised to 35.9 per cent, or more than doubled, when the amount of tobacco was reduced to half the original amount. The only difference between the two experiments was in the amount of tobacco. This indicates that the tobacco interferes in some way, probably by absorption, with the lethal effect of the gas. Hydrocyanic acid gas is lighter than air, which no doubt prevents its penetration into the hogshead in low concentrations. During the writer's experiments control beetles which were not buried in tobacco were not completely killed until 25 ounces of sodium cyanide per 1000 cu. ft. were used. The penetration of the gas increased gradually as the concentration of gas increased until that produced from 40 ounces of sodium cyanide per 1000 cu. ft. was reached, and no higher dosage was used. The penetration through tobacco at this concentration was approximately three inches. If the concentration of gas is high enough, beetles in tobacco will be killed, but the concentration for any specific fumigation depends on the depth of penetration desired and the amount of tobacco which is present for each 1000 cu. ft. to be fumigated. As warehouses are difficult to make air tight, a higher concentration than 40 ounces of hydrocyanic acid gas per 1000 cu. ft. will be necessary to give satisfactory results.

Carbon disulphide proved to be very efficient as a means of control for the tobacco beetle. Experiments tried showed a mortality of 10.30 per cent when a concentration of 8 ounces per 1000 cu. ft. was used. There was a progressive increase in the lethal effect of the fumigant until complete mortality was obtained with 32 ounces per 1000 cu. ft. This gas was apparently

not absorbed by the tobacco. On the greater lethal effect, ease of application, and non-injury to the tobacco it should prove more efficient than hydrocyanic acid gas. There is one serious handicap in the use of carbon disulphide—the fire hazard. Carbon tetrachloride was used in conjunction with the carbon disulphide as a means of reducing the fire risk. The addition of the carbon tetrachloride apparently did not increase nor decrease the toxic effect of the carbon disulphide at the concentrations used. The following statement was made by Cotton (1927). "There are a number of fumigants now on the market made up of mixtures of carbon disulphide and carbon tetrachloride. These mixtures are being used quite extensively in grain elevators and the fire risk is considered to be slight. Most of them consist of about 20% carbon disulphide and 80% carbon tetrachloride. Such a mixture is inflammable owing to the fact that the boiling point of carbon disulphide is different from that of carbon tetrachloride. To reduce this fire hazard the manufacturers saturate the mixture with sulphur dioxide. The sulphur dioxide evaporates from the mixture first and acts as a sort of fire proof blanket. This protection is of course temporary since if a can of the material were left open the protective SO_2 would soon evaporate leaving an inflammable mixture. The mixtures are toxic in proportion to the amount of CS_2 that they contain."

The toxicity of choloropicrin was tested on the tobacco beetle. Only one experiment was carried out with this gas, and that was at a concentration of 32 ounces per 1000 cu. ft. All beetles were destroyed. Chapman and Johnson (1925) have shown that chloropicrin is highly toxic to insects. They further show that this substance will retard fermentation, which would be an objectionable feature in the case of tobacco undergoing the process of aging.

Ethylene oxide was the most promising of all the fumigants used. This gas killed 78.7 per cent of beetles exposed at a concentration of 16 ounces per 1000 cu. ft., and at a concentration of 24 ounces per 1000 cu. ft. killed all beetles. The first reference to this substance as a fumigant was made by Cotton and Roark (1928). These writers state that this gas is slightly more toxic than carbon disulphide and 30 times as toxic as the vapors of carbon tetrachloride. The experiments reported in this paper support their work. In the case of carbon disulphide a total mortality was not obtained until a concentration of 32 ounces per 1000 cu. ft. had been used, whereas ethylene oxide killed all beetles at a concentration of 24 ounces. Somewhat the same condition exists as to fire hazards in the use of ethylene oxide as in the case of carbon disulphide. Back, Cotton, and Ellington (1930) state that the concentrated vapors of ethylene oxide are inflammable, but concentrations up to 3.5 pounds per 1000 cu. ft. of space are non-explosive and non-inflammable. According to this statement the danger point is well above the concentration necessary to produce total mortality, and consequently the fire risk is negligible.

Carbon dioxide has been used to increase the insecticidal efficacy of fumigants. Cotton and Young (1929) have shown that carbon dioxide in combination with various gases, accelerates the toxic action of gaseous vapors upon insects to such an extent that the dosage or length of exposure may be greatly reduced. Cotton (1930) while working with *Tribolium confusum* Duval showed that in an empty vacuum tank ethylene oxide used alone at the rate of 3.2 ounces per 100 cu. ft. of space, killed all beetles. With the tank filled with raw peanuts it required 11.2 ounces of ethylene oxide per 100 cu. ft. to kill all beetles in two hours. With the addition of carbon dioxide to the extent of 2.8 pounds per 100 cu. ft. four ounces of ethylene oxide produced a total mortality in two hours when the tank was filled with peanuts.

Jones and Kennedy (1930) make the following statement relative to the fire hazard of ethylene oxide: "to render ethylene oxide-air mixture non-inflammable under all conditions, at normal temperatures and pressures, at least 7.15 volumes of carbon dioxide per volume of ethylene oxide are required. As the molecular weight of ethylene oxide and carbon dioxide are practically the same, 7.15 pounds or more of carbon dioxide should be added to each pound of ethylene oxide to render it non-inflammable when mixed with air, and to insure a factor of safety it is recommended that at least 7.5 pounds should be used where large volumes are to be fumigated, as in grain elevators, and where it is imperative from the standpoint of safety that a strictly non-inflammable mixture be used."

To test the effect of carbon dioxide and ethylene oxide mixtures on the tobacco beetle an experiment was conducted using the substance known as carboxide, which is composed of nine parts of carbon dioxide and one part of ethylene oxide. The concentration was computed on the basis of ethylene oxide alone. Three and seven-tenths cu. ft. of the gas was run into the 8.58 cu. ft. fumigating container. This concentration was administered very slowly through the bottom inlet. All beetles were killed. Now when this concentration of ethylene oxide alone was used a mortality of only 78.2 per cent resulted. This proves that the carbon dioxide serves not only to completely remove the fire risk, but also it increases the toxicity of ethylene oxide for the tobacco beetle. Considering all of the factors involved, it is apparent that ethylene oxide is the most efficient fumigant of those tried to use for killing the tobacco beetle. The storage room should be made as tight as possible and this gas, in the form of carboxide should be administered on the basis of two pounds of ethylene oxide per 1000 cu. ft. This should give margin enough to assure satisfactory results under actual conditions.

As a practical problem it would seem possible to control the tobacco beetle. This is desirable not only from the standpoint of the tobacco manufacturer but the wholesaler, merchant and druggist as well as the general

public will benefit when the damage caused by this pest is made negligible. The wholesaler, merchant, and druggist all run the same risk of having substances other than tobacco contaminated. The damage caused can not be measured in dollars alone because the loss of good will of a customer often has a greater effect. A problem of this type is too large for a single individual or firm to solve completely, but where an infestation occurs it should be a community project, all coöperating, because a single breeding place will serve to prevent the complete control of the insect.

GENERAL SUMMARY

1. The quality of food is a determining factor in the length of life cycle of *Lasioderma serricorne* Fabr. Yeast is apparently the most satisfactory food, and the life cycle is from 18 to 20 days shorter when the beetle is fed on this substance than when it is supplied with its natural food tobacco.

2. Temperature exerts a controlling influence on the metabolic rate of the beetle. The insect's egg can exist for a period of 20 days at a temperature of 2°C. The high temperature limit which is fatal to all stages, occurs between 36°C. and 40°C. The optimum temperature is 32°C. Above and below this point the time required to complete the life cycle is lengthened. The beetle will tolerate a much wider range in temperature below the optimum than above.

3. Humidity has a definite effect on the rate of metabolism. Sustained humidities of 00, 15, 30, and 100 per cent are fatal to the beetle. The optimum is around 75 per cent. In higher and lower humidities than this the life cycle is lengthened. There is a wider range of tolerance to humidities below the optimum than above.

4. Greatest numbers of tobacco beetles complete their life cycles at humidities of about 75 per cent. Humidities above 90 per cent are unfavorable because of the attacks of fungous and bacterial diseases. The mortality at low humidities is apparently due to dessication, none developing below a humidity of 45 per cent.

5. The optimum rate of development for the tobacco beetle is near a temperature of 32°C. and in a humidity of 75 per cent, since the life cycle is shorter under these conditions in all types of food with which experiments were carried out.

6. The average number of eggs laid by a single female during her entire life (average of 100 adults) when kept under control conditions is 43.57 eggs. Results obtained from beetles caught in the storage warehouses while mating are not dependable because the insects copulate more than once.

7. The tobacco beetle will not oviposit in the absence of suitable materials upon which to lay. This holds true even if the substances are removed every other day.

8. Unfertilized female tobacco beetles will not lay eggs if copulation is prevented, though they may live two or three months. On the other hand oviposition commences soon after mating occurs, even though copulation has been delayed for a period of 10 days.

9. Physical form of materials is alone a sufficient stimulus to cause oviposition, provided the size of particles supplied is small enough. Sand, crushed glass, and sawdust passing through a No. 0000 sieve were found to cause the beetles to lay eggs. Other inorganic substances, not sized, which caused oviposition were: iron filings, wool, glass-wool, and powdered potassium dichromate.

10. Odors are a weak stimulus for inducing oviposition. Hot water extracts of the tobacco leaf are more efficient than nicotine solutions, but none of these odors approach in effectiveness the stimuli produced by natural conditions found in tobacco.

11. The beetle shows a preference for certain types or grades of tobacco. The length of its life cycle is increased: (1) by the smaller sized particles of tobacco (while it is of normal length in the coarser particles, size 00000), the length increasing in proportion to the decrease in size of sieved materials; (2) by the poorer grades of tobacco, or heavy-bodied, compact leaves.

12. There is a preference manifested for the aroma of certain types or grades of tobacco. When different kinds of tobacco of the same age are tested, the highest percentage of positive reactions is in favor of the excellent, smooth, well ripened material, while the lowest percentage is obtained from the heavy bodied grades. When the same tobacco of different ages is tested (applies only to those types that will age), the type possessing the greatest age appears to have the most acceptable aroma for the beetle.

13. The lethal effect of hydrocyanic acid gas is decreased when used in the presence of tobacco. The percentage of mortality is determined by the ratio existing between the amount of tobacco present and the concentration of the gas.

14. Carbon disulphide is an effective gas when used against the tobacco beetle. A minimum dosage of 2 pounds per 1000 cu. ft. kills all beetles under experimental conditions. The fire hazard attending this gas makes its use under practical conditions non-advisable.

15. Ethylene oxide is highly toxic to the tobacco beetle. A minimum dosage of 24 ounces per 1000 cu. ft. of space resulted in a total mortality. In combination with carbon dioxide as carboxide, in the ratio of nine parts of carbon dioxide to one part of ethylene oxide, it is absolutely non-inflammable, and the lethal effect upon the tobacco beetle is increased so that a charge of 16 ounces per 1000 cu. ft. kills all beetles. It is recommended that this gas in combination with carbon dioxide be used when tobacco warehouses are to be fumigated. The amount of gas necessary to use will depend

upon the type of storage and amount of insect damage. It is recommended that carboxide be used at the rate of two pounds of ethylene oxide per 1000 cu. ft.

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